



Proximate composition and photosynthetic pigments accumulation of soybean evaluated under different light and planting densities

Ogunboye Kehinde Dorcas¹, Olowolaju Ezekiel Dare^{2*}, Afolabi Akinjide Moses³, Adelusi Adekunle Ajayi⁴

¹⁻⁴ Botany Department, Faculty of Science, Obafemi Awolowo University, Ile Ife, Nigeria

Abstract

The objective of this study was to investigate the proximate composition, mineral nutrients and photosynthetic pigment accumulation of soybean observed under different light intensities and planting densities. The intensities of average light determined were 5764 (100%) and 3517 (61%) lux. The two treatments of light regimes were L₁=100% light, L₂= 61% light. The seeds of soybean were sown at the rate of five seeds (P₅), three seeds (P₃) and one seed (P₁). They were watered daily with 200 mL of tap water in the morning and 200 mL of tap water in the evening until they were fully established. The results obtained showed that the proximate composition such as ash, crude fibre and carbohydrate, mineral elements such as calcium and potassium, photosynthetic pigments accumulation of soybeans were more enhanced under 100% light or full light intensity while iron, protein and fat were enhanced under 61% light or partial light. Magnesium contents was similar under 61% (partial light) and 100% (full light). It can be deduced that the positive effect of the high density (P₃ and P₅) on the proximate composition, mineral nutrients and photosynthetic pigments accumulation is more than effect of the solitary (P₁). In conclusion higher densities and sufficient light is effective for improvement of proximate composition, mineral nutrient contents and photosynthetic pigments accumulation of soybean.

Keywords: density, light, minerals, nutrients, pigments, proximate

1. Introduction

Light is a source of energy for plants, required for growth and development. Light capture is critical for survivorship, growth and reproduction in plants (Yoshinaka, 2018) [17]. Higher growth and yield in plants result from either greater interception of solar radiation, higher light use efficiency, or a combination of the two (Weiner, 1990) [15]. Light interception is increased as a result of mixing two species, increasing their density and growing them together than when in pure stands (Keating and Carberry, 1993) [9]. This means that plants with higher planting density can intercept more light energy than when grown alone. Therefore, varying plant density may be a viable alternative of manipulating the productivity of crops under different environmental conditions through the changes in physiological processes (Duncan W. G 1986) [5]. Planting density promote land management and resources used, increase in yield per unit area, quality production of fruit crops, ease intercropping operation, plant protection and harvesting (Johnson, 1987) [7]. Plant density therefore is an important agronomic factor that manipulates micro environment of the field and affects growth, development and yield of crops.

Soybean is a common vegetable or legume food sources in Africa, grown as a food crop for thousands of years in China and other countries of East and South East Asia and constitutes an important component of the traditional popular diet in these regions. The seeds contain about 20% oil on a dry matter basis (Dugje *et al.*, 2009) [4], with average protein content of 40% and contain about 30% carbohydrates. The

major mineral constituents are potassium, calcium and magnesium. The minor constituents comprise of trace elements of nutritional importance, such as iron, zinc, copper. Soybean has since acquired a world-wide importance as a primary source of vegetable oil and protein. Benefits of soybean over other grain legumes commonly grown by smallholders, such as groundnut, (*Arachis Hypogea*) cowpea, (*Vigna unguiculata*), and common bean, (*Phaseolus vulgaris*), include lower susceptibility to pests and disease, better grain storage quality, a large leaf biomass, which gives a soil fertility benefit to subsequent crops and a secure commercial market for the crop (Mpeperekí *et al.*, 1996) [11]. The proximate and mineral composition of soybean has been studied by a number of researchers. Since the photosynthetic pigments accumulation elemental and nutrient composition is influenced by genetic and environmental factors, it is, therefore, imperative to evaluate other factors that could possibly enhanced photosynthetic pigments accumulation, elemental and nutritive constituents of this crop under different seeding rates and light intensities. Hence this study.

2. Materials and Methods

2.1 Experimental Seeds

The Seeds of soybean (*Glycine max* of variety TGx1740 -2F) used for this experiment were collected from the Institute of Agricultural Research and Training, (IAR and T) Ibadan, Oyo State, Nigeria. A screenhouse was constructed to minimize extraneous factors such as pests and rodents, supply of water other than the amount specifically applied. The mean daily

temperature under the screenhouse was taken with the aid of a thermometer. The intensity of light was also determined using a digital luxmeter LX 1000.

2.2 Raising of Seedlings

Seventy- two plastics pots (of 21 cm in diameter and 20 cm in depth) containing bored holes of about 3 mm each at the bottom to allow for proper drainage and prevent water logging during the course of the experiment. These pots were filled with 5 kg of collected soil. These pots were divided into two treatments of light regimes which are $L_1=100\%$ light, $L_2=61\%$ light. The seeds of the soybean were sown at the rate of five seeds P_5 , three seeds P_3 and one seed P_1 per pot. They were watered daily with 200 mL of tap water in the morning and 200 mL of tap water in the evening until they were fully established.

2.3 Determination of Proximate Composition of the Soybean Seeds

The proximate compositions (crude protein, crude fat, crude fibre and total ash) were analyzed using American association of analytical chemists (AOAC) standard methods. The total carbohydrate contents were obtained by subtracting protein, total ash, crude fat and crude protein from 100. This gave the amount of nitrogen –free extract known as carbohydrate (AOAC, 1995) [3].

2.4 Determination of Mineral Nutrient Composition (potassium (k), magnesium (Mg), calcium (Ca) and iron (Fe)) by Atomic Absorption Spectrometry:

The dried powdered samples were first digested with nitric acid and perchloric acid and then the aliquots were used for the determination of potassium, calcium, magnesium and iron content. The digested sample was analyzed for mineral content by Atomic Absorption Spectrophotometer (Perkin Elmer Analyst Model-400) in the department of Agronomy University of Ibadan, Oyo State. Different electrode lamps were used for each mineral. The amount of the energy absorbed is proportional to the concentration of the elements in the sample.

2.5 Determination of Chlorophyll Accumulation and Carotenoids

1g of soybean leaves was ground in 10 mL of 80% acetone and sodium bicarbonate using mortar and pestle. The brew was then filtered using Whatman's No 1 filter paper. The pigment quantities in the acetone extract were determined using digital spectrophotometer at wavelength 470nm, 664nm and 647nm. Chlorophyll 'a', 'b' and total chlorophyll were determined using Beer-Lambert equation as follows:

$$\text{Chlorophyll a } (\mu\text{m}) = 12.21A_{664} - 2.81A_{647}$$

$$\text{Chlorophyll b } (\mu\text{m}) = 20.13A_{647} - 5.03A_{664}$$

$$\text{Carotenoids } (\mu\text{m}) = (1000A_{470} - 3.27[\text{Chl a}] - 104[\text{Chl b}])/227$$

$$\text{Total chlorophyll } (\mu\text{m}) = 7.93 A_{664} + 19.53 A_{647}$$

In the carotenoid equation, '[chl a]' and '[chl b]' refer to the calculated concentration of chl a and chl b from the previous equations. A_{663} represent the absorbance at wavelength 663 nm while A_{646} represent the absorbance at wavelength 646 nm.

2.6 Statistical Analysis

All the data collected were subjected to Analysis of variance (ANOVA) and means were separated using Least Significant

Difference (LSD) Post-hoc at $p \geq 0.05$ level of probability.

3. Results

3.1 Mineral nutrient composition soybean at different light intensity and planting density regime

The concentration of the different nutrients, namely Calcium, Magnesium, Potassium and Iron in soybean plants were determined under different light and density treatments. The results were obtained at the harvesting stage of growth and were evaluated on a percentage basis. The nutrient concentrations among the varieties and among the treatments were presented in Table 1.

Calcium concentrations differed significantly among the light intensity regimes ($p \leq 0.05$). The L_2 plants had the highest concentration of Ca compared to L_1 plants respectively. The calcium concentration of P_3 plants was the highest while that of P_1 plants was the lowest. There was no significant difference at $p \leq 0.05$ in the calcium concentration of soybean among the planting density treatments. Among the light intensity regimes, L_1 and L_2 plants had the same concentration of magnesium. The concentrations of Magnesium were not significantly ($p \leq 0.05$) different between L_1 and L_2 plants. All the soybean plants in the different planting density had the same magnesium concentration. There was no significant difference at $p \leq 0.05$ in the magnesium contents of soybean among the density regimes.

L_2 plants had the highest concentration of potassium while L_1 plants had the lowest. There was no significant difference in potassium concentration of L_1 and L_2 plants. The potassium content of P_3 and P_5 plants were more than that of P_1 i.e. P_1 plants had the lowest concentration of potassium. There was no significant difference ($p \leq 0.05$) in the concentration of potassium among the density regimes.

Iron concentration was highest in L_1 plants and lowest in L_2 plants. The concentration of Iron in L_1 was highly significant different from L_2 plants. The iron concentration of P_3 plants was the highest while that of P_5 plants was the lowest. There was no significant difference at $p \leq 0.05$ in the level of accumulation of soybean in all the planting density treatments.

3.2 Proximate composition of soybean varieties evaluated under different light and planting densities

The result of the effect of different light intensities and planting density on the proximate composition of soybean is summarized in Table 2.

The percentage protein composition of the L_2 plants was the highest while that of L_1 plants was the lowest. There was significant difference in the protein content of L_1 and L_2 plants ($p \leq 0.05$). The percentage protein composition of P_5 plants was the highest while that of P_1 was the lowest. There was no significant difference at $p \leq 0.05$ in the percentage protein composition of soybean in all the planting density treatments.

The percentage ash content of L_1 plants was the highest while that of L_2 plants was the lowest. There was no significant difference in the percentage ash content of L_1 and L_2 plants. The percentage ash content of P_3 plants was the highest while that of P_1 was the lowest. There was no significant difference at $p \leq 0.05$ in the percentage ash content of soybean in all the planting density treatments.

The percentage fat content of the L_2 plants was the highest

while that of L_1 plants was the lowest. There was significant difference in the percentage fat content of L_1 and L_{20} plants ($p \leq 0.05$). The percentage fat content of P_5 plants was the highest while that of P_1 was the lowest. There was no significant difference at $p \leq 0.05$ in the percentage fat content of P_3 and P_5 plants but these groups were significantly different from P_1 plants at $p \leq 0.05$.

The percentage crude fibre content of the L_2 plants was the highest and that of L_{20} plants was the lowest. The percentage crude fibre of L_1 plants was significantly different from those of L_2 . But there was no significant difference in the percentage crude fibre of L_2 , L_{10} and L_{20} plants. The percentage crude fibre content of P_5 plants was the highest while that of P_1 was the lowest. There was significant difference at $p \leq 0.05$ in the percentage crude fibre content of P_1 and P_5 plants soybean, but these two groups were not significantly different from P_3 plants at $p \leq 0.05$.

The result from the present study revealed that percentage carbohydrate content of L_1 plants was the highest while that of L_2 plants was the lowest. There was significant difference in the percentage carbohydrate content of L_1 and L_2 plants. The percentage carbohydrate content of P_1 plants was the highest while that of P_5 was the lowest. There was no significant difference at $p \leq 0.05$ in the percentage carbohydrate content of soybean in the planting density treatments.

3.3 Effects of light intensities and density treatment on chlorophyll accumulation of soybean.

Table 3 showed the effect of different light intensities and planting density on chlorophyll accumulation of soybean. The results showed that L_1 plants had the highest chlorophyll a content while L_2 plants had the lowest. There was significant difference in the chlorophyll a accumulation of L_1 and L_2 plants at $p \leq 0.05$. The chlorophyll a accumulation of P_3 plants was the highest while that of P_5 was the lowest. There was no significant difference at $p \leq 0.05$ in the chlorophyll a accumulation of soybean in all the planting density treatments. The chlorophyll b accumulation of L_1 plants was the highest while that of L_2 plants was the lowest. There was significant difference in the chlorophyll b content of L_1 and L_2 plants at $p \leq 0.05$. The chlorophyll b accumulation of P_3 plants were the highest while that of P_5 was the lowest. There was no significant difference at $p \leq 0.05$ in the chlorophyll b accumulation P_1 plants and P_3 plants. The chlorophyll b accumulation of P_5 was significantly different from that of P_1 and P_3 plants at $p \leq 0.05$.

The total chlorophyll and carotenoids accumulation of L_1 plants was the highest and that of L_2 plants was the lowest. The total chlorophyll and carotenoids accumulation of L_1 plants was significantly different from those of L_2 plants at $p \leq 0.05$. There was significant difference in the total chlorophyll and carotenoids accumulation of L_1 and L_2 plants at $p \leq 0.05$. The total chlorophyll and carotenoids accumulation of P_3 plants was the highest and that of P_5 plants was the lowest. There was no significant difference in the total chlorophyll and carotenoids accumulation of soybean among the planting densities at $p \leq 0.05$.

4. Discussion

When light intensity is high, plants increase their chlorophyll

contents to lower their light compensation and light saturation points, thereby improving light adsorption and photosynthetic rate. This account for the high photosynthetic pigment accumulation of soybean which were greatest at 100% light intensity (full light) and lowest at 61 % light intensity (shade). This was in consistent with those of a previous short-term experiment on *Houttuynia cordata* (Peng, 2007) ^[12]. Therefore full light intensity in this study promote the biosynthesis of chlorophyll and carotenoid. The the highest level of chlorophyll a, chlorophyll b, carotenoid and total chlorophyll content of the soybean plants observed under three planting density can be attributed to competition for shared use of light resources accrued to this plants regimes. The lower photosynthetic pigment accumulation in the plants with only one plant density might be because the points at which competition for light becomes limiting might not have been reached probably because of lack of mutual shading effects among leaves of the solitary plants resulting in low or lack of light interception of the individual plants (Yoshinaka *et al.* 2018) ^[17].

Protein, ash and fats content of soybean generally increases under 61% light condition as observed in this study maybe as a result of low carbohydrate and crude fiber. This result agrees with those obtained in previous research on *H. cordata* (Peng, 2007) ^[12], *Moringa oleifera* (Lv, 2009) ^[10], and maize (Janaradan and Jiro, 2006) ^[8]. Therefore, as light intensity decreased, the proportion of protein, ash and fats components observed in the study plants increased. The higher ash content also observed under 100% light condition may be due to increased minerals contents to correct ionic balance under shaded condition.

The highest carbohydrates and crude fibre contents observed in the plants under 100% light condition maybe due to the buildup of assimilates and higher photosynthetic pigments accumulation which promote the composition of these attributes. This result is in agreement with Saulawa *et al.* (2014) ^[14] who obtained higher ash content, carbohydrate and crude fibre in raw baobab seed meal at high light intensity.

One expected Protein, ash, fats content, carbohydrates and crude fibre to decrease with increase in soybean population density as a result of crowding and competition but this result did not conform to this expectation, probably because of the direct manipulation of resource availability among the stands resulting in investment of resources in the tissues (Anten, 2005, 2016) ^[1, 2]. The higher Protein, ash, fats content, carbohydrates and crude fibre observed under those plants with three planting density maybe as a result of the resource capture of by an individual as influenced not only by its architecture but also by the architecture of its neighbours.

The high concentration of calcium and potassium under high light could be that the plant accumulated the calcium and potassium to deal with all injuries which happen as a result of light stress. This is in conformity with Ejaz *et al.* (2011) ^[6], who reported that the possible mechanism to minimize detrimental effect of drought in crop plant is that, the calcium level in the plant be improved. It has also been reported by Palta (2000) ^[13] that calcium and potassium are necessary for recovery from low temperature stress by activating the plasma membrane enzyme ATPase which is required to pump back the nutrients that were lost in cell damage. (Palta, 2000) ^[13].

Magnesium is involved in numerous physiological processes during plant growth and development (Waraich *et al.*, 2011) [16]. This account for the insignificant difference in the percentage composition of magnesium under different light and planting density regimes.

5. Tables

Table 1: Mineral Nutrient Composition of Soybean Evaluated under different Light and Densities Regimes

Traits	Calcium (%)	Magnesium (%)	Potassium (%)	Iron (%)
Light regime				
L ₁	0.14 ^b	0.20 ^a	1.15 ^a	5.44 ^a
L ₂	0.19 ^a	0.20 ^a	1.26 ^a	5.19 ^b
LSD	0.02	0.004	0.06	0.09
Density				
P ₁	0.16 ^a	0.21 ^a	1.18 ^a	5.19 ^b
P ₃	0.18 ^a	0.21 ^a	1.20 ^a	5.24 ^{ab}
P ₅	0.17 ^a	0.21 ^a	1.20 ^a	5.28 ^a
LSD	0.02	0.004	0.06	0.08
CV%	8.79	1.722	4.44	1.38

* Means with the same letter within the same Column are not significantly different at $P \leq 0.05$ by LSD test. L₁ = 100 % light; L₂ = 61 %; P₁ = Planting density 1; P₃ = Planting density 3; P₅ = Planting density 5; Least significant difference (LSD); CV = coefficient of variance

Table 2: Proximate composition of soybean evaluated under different light and planting densities

Traits	Protein (%)	Ash (%)	Fat (%)	Crude Fibre (%)	Carbohydrate (%)
Light regime					
L ₁	35.94 ^b	6.25 ^a	9.05 ^b	5.44 ^a	35.722 ^a
L ₂	41.02 ^a	6.23 ^a	9.66 ^a	5.19 ^b	30.982 ^b
LSD	1.43	0.19	0.089	0.09	0.09
Density					
P ₁	38.02 ^a	5.91 ^a	9.23 ^b	5.19 ^b	34.351 ^a
P ₃	38.04 ^a	5.97 ^a	9.29 ^a	5.24 ^{ab}	34.195 ^a
P ₅	38.09 ^a	5.94 ^a	9.31 ^a	5.28 ^a	34.083 ^a
LSD	1.24	0.16	0.08	0.08	1.509
CV	3.08	2.60	0.77	1.38	4.180

* Means with the same letter within the same Column are not significantly different at $P \leq 0.05$ by LSD test. L₁ = 100 % light; L₂ = 61 %; P₁ = Planting density 1; P₃ = Planting density 3; P₅ = Planting density 5; Least significant difference (LSD); CV = coefficient of variance.

Table 3: Effects of light and density treatment on chlorophyll accumulation of soybean

Trait	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoids
Light				
L ₁	18.37 ^a	28.60 ^a	51.64 ^a	5.09 ^a
L ₂	16.57 ^b	18.30 ^b	41.13 ^b	0.87 ^b
LSD	1.38	1.28	2.82	0.80
Density				
P ₁	17.67 ^a	23.78 ^a	45.04 ^a	2.76 ^a
P ₃	17.88 ^a	23.62 ^a	46.36 ^a	3.05 ^a
P ₅	17.15 ^a	21.34 ^b	44.62 ^a	2.40 ^a
LSD	1.20	1.11	2.44	0.70
CV	5.577	3.951	4.399	20.774

* Means with the same letter within the same Column are not significantly different at $P \leq 0.05$ by LSD test. L₁ = 100 % light; L₂ = 61 % light; P₁ = Planting density 1; P₃ = Planting density 3; P₅ = Planting density 5; Least significant difference (LSD); CV = coefficient of variance

Iron is an essential micronutrient required for proper photosynthesis, respiration, and other essential metabolic process in plants. The increase in iron concentration under full light may be due to the absorption and distribution of this element which is of important in plants.

6. Conclusion

In the present study, light intensity and differential planting densities impacted the mineral contents and photosynthetic pigments accumulation of soybean. However, sufficient light is essential for producing essential mineral nutrients such as calcium and potassium and some proximate composition such as ash, crude fibre and carbohydrate, all of which were enhanced under 100% light condition meanwhile iron, protein and fat were influenced by partial light (61%). Magnesium content was similar under 61% (partial light) and 100% (full light). This showed that the change of light conditions had less impact on the magnesium content. Higher planting density increased protein, fat, crude fibre and also bioavailability of minerals such as calcium, iron and potassium but it decreased carbohydrate content. In conclusion higher densities and sufficient light is effective for improvement of proximate composition, mineral nutrient contents and photosynthetic pigments accumulation of soybean.

7. References

1. Anten NPR. Optimal photosynthetic characteristics of individual plants in vegetation stands and implications for species coexistence. *Annals of Botany*. 2005; 95:495-506.
2. Anten NPR. Optimization and game theory in canopy models. In: Hikosaka K, Niinemets U, Anten N, eds. *Canopy photosynthesis: from basics to applications*. Berlin: Springer, 2016, 355-377.
3. AOAC. Association of Official Analytical Chemists. *Official Methods of Analysis 16th Edition*. The Association of Official Analytical Chemists. Virginia, Gaithersburg, MD, USA, 1995.
4. Dugje IY, Omoigui LO, Ekeleme F, Bandyopaolhyay RP, Kamara AY. *Guide to soybean production in northern Nigeria*. IITA, Ibadan, 2009, 21.
5. Duncan WG. Planting patterns and soybean yields. *Crop Science*. 1986; 26:584-588.

6. Ejaz AW, Rashid A, Ashraf MY. Role of mineral nutrition in alleviation of drought stress in plants. School of Earth and Environment, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009 Australia, 2011.
7. Johnson RR. Soybeans: Improvement, Production, and Uses, 2nd Ed.-Agronomy Monograph, 1987, 16.
8. Janaradan K, Jiro T. Alteration in intra-plant distribution of δ 15 N in response to shading in legumes. *Plant Prod. Sci.* 2006; 9:219-227.
9. Keating BA, Cardberry PS. Resource capture and use in intercropping. *Soil.* 1993; 141:119-135.
10. Lv XJ. Chance of the environmental factors is of certain influence to diurnal variation of photosynthesis and its physiological index related of *Moringa oleifera*. Huhhot: Inner Mongolia Agricultural University, 2009.
11. Mpepereki, SF, Makonese Giller KE. Soybeans in small-holder Cropping Systems of Zimbabwe. *Soil Fert Net/ CIMMYT*, Harare, Zimbabwe, 1996, 87.
12. Peng CH. Evaluation on the quality of different *Houttuynia cordata* and its regulated effect by shading treatment. Fuzhou: Fujian Agriculture and Forestry University, 2007.
13. Palta, JP. Stress Interactions at the Cellular and membrane levels. *Hort. Sci.* 2000; 25(11):1377.
14. Saulawa LA, Yaradua AI, Shuaibu C. Effect of Different Processing Methods on Proximate, Mineral and Anti Nutritional Factors content of Baobab (*Adansonia digitata*) seeds. *Pakistan Journal of Nutrition.* 2014; 13(6):314-318.
15. Weiner J. Asymmetric competition in plant populations. *Trends in Ecology and Evolution.* 1990; 5(11):360-364.
16. Waraich EA, Ahmad R, Ashraf MY, Ahmad M. Improving agricultural water use efficiency by nutrient management on crop plants. *Acta Agriculturae Scandinavica.* 2011; 61(4):291-304
17. Yoshinaka K, Nagashima H, Yanagita Y, Hikosaka K. The role of biomass allocation between lamina and petioles in a game of light competition in a dense stand of an annual plant. *Annals of Botany.* 2018; 121:1055-1064.