Ethiopian Journal of Environmental Studies & Management 13(4): 393 – 399, 2020.
ISSN:1998-0507 doi: https://ejesm.org/doi/v13i4.1
Submitted: September 7, 2019 Accepted: July 8, 2020

CHLOROPHYLL EVALUATION OF *Entandrophragma angolense* SEEDLINGS PERFORMANCE UNDER DIFFERENT LIGHT INTENSITIES AND SOIL TEXTURAL CLASSES

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Abstract

Chlorophyll is essential for photosynthesis. It serves two primary functions which is to absorb light and transfer that light energy into chemical energy. This study assessed the chlorophyll content of Entandrophragma angolense seedlings under different light intensities (25%, 50%, 75% and 100%) and soil textural classes (clay, sandyloam, sandy, loamysand and loamy). The study was conducted in a 4 X 5 factorial experiment in a Completely Randomized Design (CRD). Interactions of light intensity and soil textural classes showed that leaves of E. angolense seedlings placed under 50% light intensity grown with sandy soil had the highest chlorophyll a content of 26.4mg/l while leaves of seedlings placed under 50% light intensity grown with clay soil had the least chlorophyll content of 14.23mg/l. Interaction of light intensity and soil textural classes showed that leaf of E. angolense seedlings placed under 25% light intensity grown with clay soil had the highest chlorophyll b content of 68.82mg/l while leaves of seedlings placed under 25% light intensity grown with sandy soil had the least chlorophyll b content of 9.13mg/l. This implies that E. angolense doesn't make changes in morphological characteristics due to effect of light intensities and soil texture change in physiological characteristics such as biochemical change and example is amount of chlorophyll a and b leaf.

Key Words: *E. angolense, Light intensity, Chlorophyll, Soil textural classes, Seedlings*

Introduction

Entandrophragma angolense (Meliaceae) common name Mahogany, is most common in moist semi-deciduous forest, particularly in regions with an annual rainfall of 1600–1800 mm. However, it can also be found in evergreen forest. In East Africa, it occurs in lowland and mid-altitude rainforest, but sometimes also in gallery forest and thickets, up to 1800 m altitude. It strongly prefers well-drained localities with good water-holding capacity. The heartwood is pale pinkish brown to pale reddish brown, slightly darkening upon exposure to deep reddish brown, and distinctly demarcated from the creamy white to pale pinkish sapwood, which is up to 10cm wide. Mahogany trees have also been protected by farmers for centuries in the West African savanna, particularly where population of cattle is low and where intensive cultivation and bush fallow systems dominate. In most of the range, naturally regenerated seedlings remain the principal replacement of *E. angolense* mother trees, but as these are sought by both the local people and livestock for food and medicine (Gijsbers *et al*., 2004), an insufficient quantity remains on the forest floor to rejuvenate ageing populations. According to Kesseler (2002), under intensive cultivation, seedlings' natural regeneration no longer has a place. Kessler and Boni (2002) reported that *E. angolense* is never planted except in rare cases such as at Kokologho, Burkina Faso.

Awodoyin and Olaniyan (2007) reported that *E. angolense* is encountered in the farming systems based on varying degrees of protection against bush burning since dispersal and propagation are still at the mercy of nature, with most wild animals relishing the sweet fruit pulp. Unfortunately, most valuable tropical forest and savanna climax species, especially *E. angolense* is presently not targeted for planting programme.

Chlorophyll is a green pigment that helps absorbing sunlight for photosynthesis. Its molecule is made of a magnesium atom in a porphyrin ring. It is an essential plant pigments after seed germination when seedlings have newly emerged leaves, called cotyledon V chlorophyll. Chlorophyll allows plants to absorb energy from light which is vital for photosynthesis (Carter, 1996). Embedded in thylakoid membrane of chloroplast are chlorophyll molecules which are arranged in and around photosynthesis. Chlorophyll pigments can be separated into chlorophyll a and chlorophyll b (Marker, 1972). The identity, function and spectral properties of the types of chlorophyll in each photosynthesis are distinct and determined by each other and the protein structure surrounding them. The function center of the reaction center of chlorophyll is to absorb light energy and transfer it to other part of the photosystem (Jeffery, 1969; Gilpin, 2001). Once sunlight falls on plant leaves, photons is absorb by chlorophyll in two photosystems and sunlight energy absorbed by chlorophyll contributes in splitting water molecule where oxygen molecule is produced. This process is light reaction of photosynthesis process that produces energy and completed by dark reaction photosynthesis product which are glucose and oxygen.

Chlorophyll is present abundantly in nature and, due to its critical "light harvesting'' role in photosynthesis, is vital to the survival of both the plant and animal kingdoms (Humphrey, 2004). Chlorophyll selectively absorbs light in the red and blue regions and therefore emits a green colour. Photosynthesis is a process which uses this harvested light energy together with water and carbon dioxide to produce oxygen and carbohydrates; as such, it converts solar energy into chemical energy. The products from this chemical process reflect its significance, with carbohydrates being the primary building block for plants and oxygen being necessary for the survival of animal kingdom (Humphrey, 2004]. The importance of photosynthesis for life on earth is further highlighted by plants forming the basis of all food chains. Chlorophyll is a compound that is decomposed and reproduced continuously in significant amounts both terrestrially and in the oceans. It is estimated that 1.2 billion tons of chlorophyll are produced annually in the planet (Humphrey, 2004).

Chlorophyll is used as a colouring agent due to its selective absorbance of light of certain wavelengths and its consequent green colour. Changes in market demands and legislation have resulted in the requirement of natural colouring agents to be used in food products in preference to artificial colourings (Spears, 1988]. Colouring is essential for both consumers and manufacturers, as many foods lose their original colours due to chemical processes they undergo. Consumers demand products of original appearance, while manufacturers desire uniformity for all products (Spears, 1988 and (Timberlake *et al.,* 1986). Chlorophyll in plants is confined in chloroplasts where it is not only complexed with phospholipids, polypeptides and tocopherols but also protected by a hydrophobic membrane (Humphrey, 2004). When chlorophyll is removed from this protective environment, its magnesium ion becomes unstable and may easily be displaced by a weak acid. In order to overcome this problem, the magnesium ion is often substituted with a copper ion to form a highly stable blue/green complex (Humphrey, 2004 and Timberlake *et al.,* 1986).

Environmental conditions to a large extent determines the survival and growth of seedlings within a forest (Tilman, 1986). The sensitivity of plants to light quality and quantity plays a vital role in their physiological development (Aphalo and Ballare, 1995). The degree of shade created by the canopy is a key parameter that determines the amount of radiant energy available for photosynthesis in growing seedlings (Perry, 1994). Soil type is also a key parameter in seedling survival and growth because of the sensitivity of photosynthesis to water available in the soil (Jones, 1992). Hence, study will aid the selection of the most appropriate light intensity and best soil textural class that will facilitate chlorophyll content production for optimal seedling growth performance of the species. Due to the importance of this species, information on how to propagate this species could be of great value for reforestation effort in different parts of Nigeria.

Materials and Method

The study was carried out at the Tree Improvement Nursery and Silviculture Nursery of the Department of Sustainable Forest management, Forestry Research Institute of Nigeria, Jericho Hill, Ibadan, Nigeria (FRIN). FRIN is located within longitude 07°23'18"N to 07°23'43"N and latitude 03°51'20"E to 03°51'43"E. Mean annual rainfall is about 1548.9 mm, falling within approximately 90 days. The mean maximum temperature is 31.9°C, minimum 24.2°C while the mean daily relative humidity is about 71.9%. The experimental design used for this study was 4 × 5 factorial experiment in completely randomized design. Factor A: 4 light intensity and Factor B: 5 textural classes of soil which constituted the treatments. Each textural class of soil was replicated 10 times.

For chlorophyll extraction, the sample of leaves were collected from each treatment and prepared to determine their chlorophyll content. This was determined by the method of Bolanle-Ojo *et al*. (2018). 1.5 g was removed from the leaves collected from each treatment with the aid of a weighing balance. The leaf samples were grinded with 80 % methanol (Bolanle-Ojo *et al.,* 2018) with the aid of pestle and mortar. The residue was removed from the liquid with the aid of a filter paper. Methanol (80 %) was used to blank the meter after which the extract was introduced into the spectrophotometer. The readings from the meter were used to determine the chlorophyll concentrations using the following equations formulated by Arnon (1949) in Bolanle-Ojo *et al*. (2018);

$Ca = 12.7A_{663} - 2.69A_{645}$ ……… (ii)

$Cb = 22.9A_{645} - 4.68A_{663}$……… (iii)

D645 = Absorbance at 645nm (chlorophyll a)

D663 = Absorbance at 663nm (chlorophyll b)

Data Analysis

Data collected were subjected to analysis of variance and means found to be different were separated using Duncan Multiple Range Test procedure.

Results and Discussion

Effect of light intensities on the chlorophyll a of leaf of E. angolense seedlings

The mean chlorophyll a content of leaf of *E. angolense* seedlings ranged from 20.34 to 20.70 mg/l. Leaves of seedlings placed under 75% Light intensity (LI) had the highest chlorophyll a content while leaves of seedlings placed under 100% LI had the least (Table 1). Mean separation for light intensities showed that chlorophyll a content of leaves of *E. angolense* seedlings placed under different light intensities were significantly different (p≤0.05) from each other (Table 1).

ANOVA revealed significant effect (p≤0.05) of light intensities on the Chlorophyll a content of *E. angolense* seedlings leaves (Table 4).

Effect of light intensities on the chlorophyll b of leaf of E. angolense seedlings

ANOVA revealed that there was a significant effect (p≤0.05) of light intensities on the Chlorophyll b (Table 4). For light intensities, the mean chlorophyll b content of leaf of *E. angolense* seedlings ranged from 16.63 to 44.49 mg/l. Leaves of seedlings placed under 25% Light intensity (LI) had the highest chlorophyll b content while leaves of seedlings placed under 100% LI had the least (Table 1). Mean separation for light intensities showed that chlorophyll b content of leaves of *E. angolense* seedlings placed under different light intensities were significantly different (p≤0.05) from each other (Table 1)

Effect of soil textural classes on the chlorophyll a of leaf of E. angolense seedlings

The mean chlorophyll a content of leaf of *E. angolense* seedlings ranged from 19.87 to 21.63 mg/l. leaves of seedlings grown with sandy soil had the highest chlorophyll a content while leaves of seedlings grown with clay had the least (Table 2). Mean separation for soil textural classes showed that chlorophyll a content of leaf of *E. angolense* seedlings grown with different soil types were significantly different (p≤0.05) from each other (Table 2). ANOVA revealed significant effect (p≤0.05) of soil textural classes on the Chlorophyll a content of *E. angolense* seedlings leaves (Table 4).

Effect of soil textural classes on the chlorophyll b of leaf of E. angolense seedlings

ANOVA revealed that there was a significant effect (p≤0.05) of soil texture on Chlorophyll b (Table 4). The mean chlorophyll b content of leaf of *E. angolense* seedlings ranged from 14.57 to 44.00 mg/l. leaves of seedlings grown with clay soil had the highest chlorophyll b content while leaves of seedlings grown with sandy soil had the least (Table 2). Mean separation for soil textural classes showed that chlorophyll b content of leaf of *E. angolense* seedlings grown with different soil textural classes were significantly different (p≤0.05) from each other (Table 2).

Effect of interaction of light intensities and soil textural classes on the chlorophyll a of leaf of E. angolense seedlings

Interactions of light intensities and soil textural classes showed that leaves of *E. angolense* seedlings placed under 50% LI and grown with sandy soil had the highest chlorophyll a content of 26.4 mg/l while leaves of seedlings placed under 50% LI and grown with clay soil had the least chlorophyll a content of 14.23 mg/l (Table 3). Mean separation for the interaction of light intensities and textural classes revealed that the chlorophyll a content of leaves of *E. angolense* seedlings under treatments L1S4, L1S5, L2S5 and L4S3 were not significantly different from each other. Results showed that effects of treatments L1S5, L3S2 and L4S3 on chlorophyll A were not significantly different (P > 0.05) from each other. Chlorophyll synthesis from seedlings under treatments L1S1, L1S2, L3S4, L4S1, L4S2 and L4S5 were not significantly different from each other (Table 3).

ANOVA revealed significant effect (p≤0.05) of the interaction of light intensities and soil textural classes on the Chlorophyll a content of *E. angolense* seedlings leaves (Table 4).

Table 1: Mean Separation for the Effect of Light Intensities on the Chlorophyll a and b of Leaves of *E. angolense* Seedlings

Light Intensity	Chlorophyll a	Chlorophyll b
100%	20.34 ±0.02^{c}	16.63 ±0.01^{d}
75%	20.70 ±0.02^{a}	30.52 ±0.01^{b}
50%	20.60 ±0.02^{b}	25.78 ±0.01^{c}
25%	20.66 ±0.02^{a}	44.49 ±0.01^{a}

Means±SE with same alphabet in each column are not significantly different (p≤0.05)

Table 2: Mean separation for the Effect of Soils on Chlorophyll a and b of Leaves of *E. angolense* Seedlings

Soils	Chlorophyll a	Chlorophyll b
Clay	19.87 ± 0.02^{e}	44.00 ± 0.01^{a}
Sandyloam	20.47 ± 0.02^{c}	34.14 ± 0.01^{b}
Sandy	21.63 ± 0.02^{a}	14.57 ± 0.01^{e}
Loamysand	20.67 ± 0.02^{b}	26.88 ± 0.01^{d}
Loam	20.25 ± 0.02^{d}	27.17 ± 0.01^{c}

Means±SE with same alphabet in each column are not significantly different (p≤0.05)

Table 3: Mean Separation for the Interaction Effect of Light Intensities and Soils on the Chlorophyll a and b of Leaves of *E. angolense* Seedlings

L * S	Chlorophyll a	Chlorophyll b
L1S1	20.96 ± 0.04^{f}	17.26 ± 0.01^{k}
L1S2	20.18 ± 0.04^{f}	15.96 ± 0.01^{l}
L1S3	24.25 ± 0.04^{b}	16.43 ± 0.01^{kl}
L1S4	17.75 ± 0.04^{h}	20.71 ± 0.01^{j}
L1S5	18.57 ± 0.04^{gh}	12.81 ± 0.01^{m}
L2S1	23.77 ± 0.04^{c}	37.33 ± 0.01^{f}
L2S2	21.96 ± 0.04^{de}	42.88 ± 0.01^{d}
L2S3	17.4 ± 0.04^{h}	21.94 ± 0.01^{i}
L2S4	22.44 ± 0.04^{d}	39.15 ± 0.01^{e}
L2S5	17.92 ± 0.04^{h}	11.28 ± 0.01^{n}
L3S1	14.23 ± 0.04^{i}	58.57 ± 0.01^{b}
L3S2	18.99 ± 0.04^{g}	15.27 ± 0.01^{l}
L3S3	26.4 ± 0.04^{a}	10.77 ± 0.01^{n}
L3S4	20.17 ± 0.04^{f}	15.42 ± 0.01^{l}
L3S5	23.24 ± 0.04^{c}	28.86 ± 0.01^{h}
L4S1	20.51 ± 0.04^{f}	62.82 ± 0.01^{a}
L4S2	20.77 ± 0.04^{f}	62.47 ± 0.01^{a}
L4S3	18.44 ± 0.04^{gh}	9.13 ± 0.01^{o}
L4S4	22.33 ± 0.04^{d}	32.26 ± 0.01^{g}
L4S5	21.27 ± 0.04^{ef}	55.75 ± 0.01^{c}

Means±SE with same alphabet in each column are not significantly different (p≤0.05)

Where: L1 = 100%, L2 = 75%, L3 = 50%, L4 = 25%, S1 = Clay, S2 = Sandy-loam, S3 = Sandy, S4 = loamy-sand and S5 = loam

Effect of interaction of light intensities and soil textural classes on the chlorophyll b of leaf of E. angolense seedlings

ANOVA revealed that there was a significant interaction effect (p≤0.05) of light intensities and soil textural classes on the Chlorophyll b (Table 4). Interactions of light intensities and soil textural classes showed that leaf of *E. angolense* seedlings placed under 25% LI and grown with clay soil had the highest chlorophyll a content of 62.82 mg/l while leaf of seedlings placed under 25% LI and grown with sandy soil had the least chlorophyll a content of 9.13 mg/l (Table 3). Mean separation for the interaction of light intensities and soil textural classes revealed that the chlorophyll b content of leaf of *E. angolense* seedlings under L1S2, L1S3, L3S2 and L3S4 were not significantly different from each other. Chlorophyll synthesis from seedlings under treatments L1S1 and L1S3 were not significantly different from each other. The same trend was observed in seedlings from treatments L4S1 and L4S2 which were not significantly different from each other while other treatment combinations were significantly different from each other (Table 3).

Table 4: ANOVA for the Effect of Light Intensities and Soil Types on the Chlorophyll a and b of leaves of *E. angolense* Seedlings

Variable	SV	Df	SS	MS	F	Sig.
Chlorophyll a	LI	3	1.17	0.39	103.92	0.00*
	S	4	20.78	5.19	1389.51	0.00*
	LI * S	12	430.21	35.85	9589.95	0.00*
	Error	40	0.15	0.00		
	Total	59	452.30			
Chlorophyll b	LI	3	6073.31	2024.44	4671777.12	0.00*
	S	4	5602.10	1400.53	3231980.30	0.00*
	LI * S	12	7788.58	649.05	1497804.19	0.00*
	Error	40	0.02	0.00		
	Total	59	19464.01			

*significant at (p≤0.05)

Discussion

Chlorophyll a and b content of leaf of *E. angolense* seedlings under low light intensities were higher compared to the leaf of seedlings under 100 % light intensity. This is in correlation with the work of Bolanle-Ojo (2014) who report that low light intensities increased the chlorophyll a and b content of leaf of *A. heterophyllus* seedlings after four (4) months of exposure to different light intensities. Chlorophyll a was higher in other soil textural classes expect for clay that had the lowest while chlorophyll b was higher in clay soil compared to other soil types.

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