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An Assesesment of Leaf Chlorophyll Concentration of Afforestation Tree Species in South-Eastern, Nigeria

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Abstract

Leaf chlorophyll content provides valuable information about physiological status of plants. However, fewer studies have investigated the difference in chlorophyll concentration in leaves of tropical afforestation tree species. Therefore, this study examines the difference in foliar chlorophyll contents of six tropical afforestation tree species namely: *Tectona grandis, Pentaclethra macrophylla, Piptademiastrum africanum, Azadirachta indica, Brachystegia eurycoma* and *Gmelina arborea* found in the relict forest in Umudike, South east, Nigeria. A single factor experiment in a completely randomised design in three replicates was employed to analyse the rate of leaf chlorophyll contents. Fisher's least significant different was used to test for significance in mean difference in foliar chlorophyll contents between tree species at 95% confidence interval using analysis of variance. The results of this study showed a significant difference in foliar chlorophyll concentration between the tree species with *Tectona grandis* having a higher chlorophyll concentration than other trees this could be as a result of its higher vegetative activity which increases its primary productivity followed by *Pentaclethra macrophylla* while *Azadirachta indica* having least the chlorophyll concentration. The study further revealed that other indigenous tree species like *Piptademiastrum africanum* and *Brachystegia eurycoma* have higher chlorophyll concentration. Further studies should be carry out to examine factors that have contributed informed the differences in the chlorophyll concentration of these trees species, thus this would broaden the understanding of their physiological status and equally encourage there conservation.

Key Words: physiological status, chlorophyll concentration, tree species, tree foliar, environmental stress, productivity

Introduction

Chlorophyll is a key indicator of the physiological status of forest canopy (Curran et al. 1990; Curran et al. 1995). However, the chlorophyll content of a forest canopy can be an indicator of such measures of physiological status as photosynthetic capacity, developmental stage, productivity, and stress (Ustin et al. 1988; Curran et al. 1990; Richardson et al. 2002; Khaleghi et al. 2012). Chlorophyll is the green pigment in tree leaves which initiates photosynthesis by absorbing energy from sunlight and transfers this energy to other molecules (Lei et al. 1996; Spear 1997; Ekanayake et al. 2004). Thus, this energy is used to synthesis carbohydrate from CO_2 and water (Khaleghi et al. 2012). Chlorophyll is of two types' chlorophyll a and chlorophyll b which both of them works as photoreceptor in pho-

tosynthesis (Falk et al. 1996; Khaleghi et al. 2012). The chlorophylls Chl a and Chl b, are the most important of these pigments, and are thus virtually essential for the oxygenic conversion of light energy to the stored chemical energy the powers the biosphere (Richardson et al. 2002; Oyetunji et al. 2007). In addition, Oyetunji et al. (2007) stated that chlorophyll content is also used to determine light use efficiency for photosynthesis which occurs at cellular level. The intensity of chlorophyll fluorescence is directly related to the concentration of excited chlorophyll molecules, which suggests that a change in the fluorescence yield be related to a change in the efficiency of photosynthesis, therefore, providing a measure of leaf photosynthetic ability of plants (Curran et al. 1995; Larcher 1995; Oyetunji et al. 2007; Richardson et al. 2002; Khaleghi et al. 2012).

From the applied perceptive, leaf pigmentation is important to both land managers and ecophysiologists (Richardson et al. 2002). According to Curran et al. 1990; Curran et al. 1995; Filella et al. 1995; Richardson et al. (2002), these are informed by several factors, first, the amount of solar radiation absorbed by a leaf is largely a function of synthetic pigments, and therefore low concentrations of chlorophyll can directly limit photosynthetic potential and hence primary production. Secondly, much of leaf nitrogen is incorporated in chlorophyll, so quantifying chlorophyll gives an indirect measure of nutrient status in plants (Filella et al. 1995; Moran et al. 2000). Thirdly, pigmentation can be directly related to stress physiology, as concentrations of carotenoids increase and chlorophylls generally decreases under stress and during senescence (Penuelas and Filella 1998). Fourthly, the relative concentrations of pigments are known to change with abiotic factors such as light (eg sun leaves have Chl a: Chl b ratio) (Larcher 1995) and so quantifying these proportions can provide important information about relationship between plants and their environment (Richardson et al. 2002; Heschel et al. 2014).

Thus, chlorophyll is at the centre of photosynthesis oxidation − reduction reaction between carbon (IV) oxide and water (H_2O) (Richardson et al. 2002; Curran et al. 1990; Curran et al. 1995). When chlorophyll absorbs light energy the molecules enters into a higher energy state in which it easily gives up an electron to the first available electron-ac-

cepting molecules nearby (Flagella et al. 1995). Leaf chlorophyll probe is a powerful and sensitive intrinsic measurement of the photosynthetic process (Oquist and Wass 1988), which can be used to detect the influence of various environmental stress factors (Oyetunji et al. 2007; Richardson et al. 2002; Flagella et al. 1995).

However, in photosynthesis, antenna pigments in leaf chloroplasts absorbs solar radiation, and through resonance transfer the resulting excitation is channelled to the reaction centre pigment, which release electrons and sets in motion the photochemical process (Richardson et al. 2002).

Although, other factors contribute immensely to primary productivity of trees in relation to foliar chlorophyll concentration such as amount of leaves (leaf structure characteristics), water use efficiency, fresh and dry weight of tissue per unit leaf area and some underlying physiology of the plant- nitrogen nutrition, growth environment (eg. Shade plant growth in sun, pioneer plant grown in partial shade). This study is own focusing on the physiology characteristic of these afforestation tree species using only leaf chlorophyll as a treatment.

The tree species used in these studies included, Tectona grandis Linn. F (Teak), *Piptademiastrum africanum* Benth, *Azadirachta indica* A. Juss, *Pentaclethra macrophylla* (Hook. F). Brenan, *Brachystegia eurycoma* Harms and *Gmelina arborea* Roxb found in relict forest of Umudike. Relict forest are forest which still in exist as a remnant of a widely distributed forest, even though the environment in which the originally develop has been modify due to changing human landscape. More so, in this study a trees is define base on the height if tree is less than 6meters is categorised as a shrub and greater than 6 meters it is referred as a tree (Keay et al. 1964; Hutchinson and Dalziel 1972; Keay 1989; Ekukudo et al. 2001; Ekukudo 2003). However, these are tropical multi-purpose trees which have numerous economics and ecological benefits. More so, these tree species are highly preferred for their timber quality, non-timber forest products (NTFPs) and their regulating, provisioning and supporting ecosystem services (Borokini 2014a, 2014b; Langenberger and Liu 2013; Hashmat et al. 2012; Ekukudo et al. 2001; Ekukudo 2003; Keay 1989; Hutchinson and Dalziel 1972; Keay et al. 1964). However, information on the leaf physiological status could give more insight to plant physiologist to identify which tree species has higher pig-

ment than other and which could related to their ability to withstand environmental stress. Richardson et al. (2002) stated that from physiological perceptive, leaf chlorophyll content, for example varies both between and within species and is therefore a parameter of significant interest in its own right. However, information on the physiological status of most tropical afforestation tree species are limited with regards to their multiple values and environmental sustainability role the play.

Thus, the rationale for this research is to compare the difference in leaf/foliar chlorophyll concentrations of some tropical afforestation tree species which are commonly found in South Eastern, Nigeria. This could act as an incentive for physiologist to further examine other characteristics of these trees species in other to improve their economic and ecological functions and equally sustain their conservation.

Materials and Methods

Study Area

The study was conducted at a relict forest in Umudike, South Eastern, and Nigeria. Umudike is located in Abia State on latitude $05^0, 28^1$N and longitude $07^0, 32^1$E. The average elevation is 119 metres. The rainfall pattern is bimodal in distribution with an annual rainfall of between $2,000 \sim 3,000$ mm (Korieocha et al. 2011; John et al. 2014). The rainy season starts from March to October while the dry season starts from November to March when the North East trade (harmattan) wind weeps across the entire area. However, the commencement and cessation of rainfall had dramatically changed due to changing climate and can no longer be predicted as usual. These changes are becoming very obvious recently (the past five years). The ambient temperature is between 24^oC to 32^oC and relative humidity ranges from $60 \sim 80\%$. The area has lateritic soil, which is generally characterised by excessive leaching, resulting in the loss of soil nutrients. The vegetation of the area is that of tropical rain forest in formation but due to unguided agricultural expansion and conversion pressure, the area has dramatically been reduced to shrubs and grass vegetation or derived savannah.

Sampling Techniques

The tree species were selected based on the two principal

groups of tropical tree species in the relict forest, the climax (shade tolerant) and pioneer (or light demanding) and exotic and indigenous (Keay et al. 1964; Hutchinson and Dalziel 1972; Denslow 1987; Swaine and Whitmore 1988; Keay 1989; Whitmore 1999). Out of the tree species selected three were pioneers (exotic) Tectona *grandis* Linn. F (Teak), *Azadirachta indica* A. Juss and *Gmelina arborea* Roxb and were introduced into Nigeria during colonial era because of their preferred timber, non-timber, aesthetic values and invaluable environmental services the provide while the remaining were climax species (indigenous) and leguminous with great importance in fixation nitrogen in the soil which improves soil fertility (Udeagha 2008). In addition, these tree species are widely used for afforestation programmes in Nigeria because of their very high level of adaptation to adverse environmental changes (Keay1989; Ekukudo et al. 2001; Udeagha 2008; Ekukudo 2003; Borokini 2014a), thus their physiological status are not well documented.

The leaves of the six tree species were randomly selected from a relict forest of about 50 years (Latitude N 05^0 28. 695' and Longitude E 007^0 32. 471') umudike, which has been undergoing modification due consistent human interference. Leaves of trees of 6 meters and above were collected and used for the laboratory analyses. Three leaves were collected from each trees per specie in the same place to ensure randomisation and representativeness of the tree species in the relict forest. In all a total of 108 leave samples were collected used for the experiment. This study also adopted some of the approaches used by Curran et al. (1990), Curran et al. (1995) and Khaleghi et al. (2012) in arriving at the sample size. The experiment was repeated three (replicate) times in order to minimise sample error (Groves 2004).

Laboratory Analyses

Determination of Chlorophyll contents

Leaf chlorophyll content was determined using the method described by Arnon (1949); Ekanayake and Adeleke (1996) and Phoguodume et al. (2012). Six fully expanded upper leaves of the trees were collected and used for extraction of chlorophyll. Leaf tissue weighing 2 g was crushed in a mortar and 80% acetone added to it in sufficient quantities to allow the tissue to be thoroughly homo-

genized. The supernatant was decanted through a filter paper into a 100 ml volumetric flask. Acetone was again added to the residue in the mortar and the extraction procedure repeated. Additional acetone was used to wash off the chlorophyll until the 100 ml mark was attained in the volumetric flask. The resulting solution was thoroughly mixed and 5 ml pipetted into a 50 ml volumetric flask and made up to volume with 80% acetone. The extracts were kept in the dark at 4°C for seven days. The absorbance of the extract was then measured at 645, 663, and 652 nm wavelengths for chlorophyll a, b and ab, respectively, using model 690 spectrophotometer with 80% acetone been used as blank. The experiment was carryout at the Biochemistry Laboratory, College of Biological and Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria. Unlike other methods which use solvents like Dimethyl Formamide (DMF) and Dimethyl Sulfoxide (DMSO), the use of acetone in this method was because it is not toxic to human and not easily absorbed by the skin (Dae Park et al. 2012). Concentrations of chlorophyll (mg/g fresh leaf weight) were calculated as follows: chlorophyll a=$(20.2x D_{645})x(50/1000)x(100/5)x(1/2)$; chlorophyll b=$(8.02xD_{663})x (50/1000)x(100/5)x(1/2)$; and total chlorophyll=$(20.2xD_{645} +8.02xD_{663})x(50/1000)x(100/5)x(1/2)$, where D=absorbance (Anon (1949), Ekanayake and Adeleke (1996) and Phoguodume et al. (2012).

Statistical analysis

The mean values were computed for each tree and the variations in the mean tree chlorophyll were evaluated through analyses of variance (ANOVA) ($p < 0.05$), while Fisher Least Significant Difference (LSD) was applied for separation of significantly different means of foliar chlorophyll difference per tree at 95% confidence interval.

Results and Discussion

Chlorophyll concentration

The outcomes of statistical analyses done on the data from the experiments conducted to examine the difference in foliar chlorophyll concentration (Table 1) for the mean separation test showed that *T. grandis* has a higher chlorophyll concentration among other tree species investigated

Chlorophyll concentration

Chlorophyll a

Chlorophyll a concentrations of all the tree species are significantly different from each other. *T. grandis* had the highest chlorophyll concentration with *A. indica* having the lowest chlorophyll a concentration.

Chlorophyll b

Significant differences occurred in chlorophyll b concentration among all the tree species. *T. grandis* and *P. macrophylla* have the highest concentration of chlorophyll b but were statistically different from each other ($p < 0.05$) (Table 1). *B. eurycoma* and *A. indica* concentration are not statistically significant from which other while *G. arborea* had the lowest chlorophyll b concentration among the entire tree species investigated. Lahai et al. (2003) explained that the variation observed in chlorophyll b concentration could be due to increase in environmental stress experienced due to extremities in weather events. A similar phenomenon was reported by Carter (1994); Craig and Shih (1998) and Sari et al. (2005) that extreme weather events increased stress conditions for nutrition and some other stress conditions which cause the reduction in photosynthetic activities and chlorophyll contents in the trees (Filella et al. 1995; Moran et al. 2000). This could have been the reason for low chlorophyll content in *G.aborea* and *A. indica* (Curran et al. 1990; Filella et al. 1995; See Richardson et al. 2002). On the oth-

Table 1. Mean chlorophyll concentration in the leaves of tree species for chlorophyll a, chlorophyll b and chlorophyll ab

Species	Chlorophyll Concentration ($\mu mol/g^2$)		
	Chl a	Chl b	Chl ab
Tectona grandis	1.6470[a]	1.6400[a]	3.2864[a]
Piptadeniastrum africanum	1.4850[b]	1.2311[b]	2.7158[c]
Pentaclethra macrophylla	1.4140[c]	1.4958[c]	2.9097[b]
Brachystegia eurycoma	0.8690[d]	0.8140[d]	1.6826[d]
Gmelina arborea	0.6363[e]	0.5614[e]	1.1977[e]
Azadirachta indica	04141[f]	0.8301[d]	1.2442[d]

Chl a, Chlorophyll a; Chl b, Chlorophyll b and Chlorophyll ab. [a,b,c,d,e,f]Means with different superscripts (letters) are significantly different from each other, means followed by the same superscripts are not significantly different using FLSD ($p < 0.05$).

er hand, the high concentration of chlorophyll b which enhances the utilization of carbon (IV) oxide to produce carbohydrate in *T.grandis* could be attributed to its ability to withstand extreme weather events than other tree species examined (Larcher 1995; Falk et al. 1996; Carter and Knapp 2001; Richardson et al. 2002; Khaleghi et al. 2012; See Heschel et al. 2014). This confirmed with the findings of Ekanayake et al. (2004), Pervial and Sheriffs (2002), Flagella et al. (1995) that chlorophyll concentration provides rapid and accurate techniques of detecting and quantifying plant tolerance to stress. This implies that trees with higher foliar chlorophyll content could have the ability to withstand adverse weather events as would increase their adaptive capacity.

Chlorophyll ab

T. grandis was significantly different from all other tree species, in terms of chlorophyll ab concentration. *A. indica* and *B. eurycoma* are significantly similar to each other (Table 1) with *G. arborea* having the lowest chlorophyll ab concentration (Table 1). The reason for the low foliar chlorophyll concentration in some of the tree species investigated could be due to decrease in photosynthetic activity paralleled by a reduction in leaf chlorophyll content which affects crop and tree performance (Curran et al. 1990; Filella et al. 1995; Ekanayake et al. 1998; Richardson et al. 2002; Ekanayake et al. 2004; Heschel et al. 2014). This implies that the foliar chlorophyll content of tree species play important role in enhancing the primary productivity of trees. Tree species with higher concentration of chlorophyll could have the tendency to grow faster and sequester more carbon that would enhance photosynthetic activities in the trees (See Richardson et al. 2002; Khaleghi et al. 2012). However, Langenberger and Liu (2013), noted that teak are perceived to have high timber value because of it fast growth rate, which make it be a suitable complement in smallholder land management systems diversifying production and income base for farmers. According Clark et al. (2000) functional state of photosynthesis has been considered as an ideal to monitor the health and vitality of plants. Gitelson et al. (2003) also stated that leaf chlorophyll content provides valuable information about physiological status of plants.

Conclusion

The study indicates that significant difference ($p < 0.05$) occurred between all the tree species for the parameters investigated. However, the understanding of spectral features of tropical trees is important in order to determine their physiological activities in respect to photosynthetic function and primary productivity as this could assist in understanding the physiological status of most afforestation tree species in Nigeria. Although the leaf area varies per tree, the study shows that leaf area does not actually influence the foliar chlorophyll concentration in the trees investigated. For instance, *T. grandis* has largest leaf area followed by *G. arborea* which in this case has the lowest chlorophyll concentration while other trees with small leaf area (*P. africanum*, *B. eurycoma*, *P. macrophylla*, among others) have higher chlorophyll concentration. Further studies should be carry out to examine factors that have informed the differences in the chlorophyll concentration of the trees species, thus this would broaden the understanding of their physiological status and equally encourage there conservation.

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