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Effect of Light Intensity and Orientation on In-Vitro Regeneration of Cassava Plantlets

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Abstract: *Replacement of white fluorescent tube (FL) with compact fluorescent light (CFL) as an alternative source of light for tissue culture in developing countries like Nigeria was evaluated. The effect of different CFL bulbs with photon flux densities (PFD) of 23, 41, 51 and 184 µMol $m^{-2}s^{-1}$arranged in horizontal, angular and vertical orientations on plant height, leaf node formation, root proliferation and plantlets regeneration were investigated. All four factors were differently affected by light intensity and orientation. Plantlet regeneration was positively impacted by light intensity of 51 µMol $m^{-2}s^{-1}$and by angular orientation, plant height and root proliferation were optimum at light intensity of 184 µMol $m^{-2}s^{-1}$and vertical orientation respectively, while differences in light intensities and orientation had no significant impact on leaf node formation. Light intensities of 51 µMol $m^{-2}s^{-1}$ and angular orientation were adopted as optimal for all four factors considered.*

Keywords*: Tissue culture, Light intensity, Light orientation, Photon flux density, plantlet regeneration*

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I. Introduction

Plant tissue culture is a valuable tool in achieving improved and increased agricultural productivity in sub-Sahara Africa. Some of its benefits include the production of exact copies of plants with desirable traits (clonal propagation) and the rapid production of matured plants. It has been used in the production of disease free planting materials and has facilitated the transport of plant across borders with minimal risk of disseminating diseases or pests. The application of this technology, has also aided the rescue of embryos from seeds with limited chances of germination and survival, as well as served as a platform for the regeneration of genetically modified and genome edited plants. Despite its enormous importance, establishment of tissue culture laboratories in developing countries like Nigeria is not as wide spread as in developed countries. Very few institutions and private enterprise can afford to establish and efficiently run tissue culture laboratories in the country. This could be attributed to power instability and the availability of other intrinsic factors that govern the successful establishment of *in-vitro* plantlets. *In-vitro* plant propagation requires adequate culture medium comprising of macro and micronutrient, organic nitrogen and carbon source and of course plant growth regulators. It also requires optimum incubation environment with regulated temperature, humidity, light source and photoperiod [1].

Light is the most essential factor controlling plant growth, morphogenesis,metabolism and chlorophyll content of plant cell, tissue and organ cultures [3,4].Itacts as a signaling mechanism through different light receptors and provides the required energy for plant growth and development through photosynthesis[4]. The quality of light (spectral) essential for plant growth and morphogenesis commonly referred to as photosynthetically active radiation (PAR)falls between 400 and 700 nm wavelength and is matched to plant photoreceptors for optimum production, enhanced morphology and chemical composition [5]. Likewise, light quantity (intensity) and duration (photoperiod) have been proven by various studies as also essential for photosynthesis, photomorphogenesis and phototropism [6, 7]. This implies that the development and performance of *in-vitro* plantlets can be improved by changing the light quality, quantityand duration in the growth environment [8]. The effect of light source and intensity on different *in-vitro* growth parameters such as shoot regeneration, plant height and size, fresh weight, chlorophyll and carotenoid contents of leaflets have been demonstrated in variety of crops including potato [7], sugarcane [9], citrus [10], bananas [11] and cauliflower [12].Although natural sunlight could be used as energy source for *in-vitro* propagation of plants, artificial light sources such as fluorescent lamps (FL), metal halide, high pressure sodium, incandescent lamps [13]and light emitting diodes (LED) [1]are mostly used in commercial tissue culture laboratories. This is to provide the requisite axenic environment essential for excluding microbes and other contaminants that could inhibit the growth of *in-vitro* propagated plantlets. The white fluorescent is the most widely used light source in most tissue culture laboratory worldwide. It is a low pressure mercury-vapor gas-discharge lamp that uses fluorescence to

produce visible light and has a control gear system consisting of choke and starter. The use of FL as a light source in tissue culture laboratories in Nigeria was inefficient due to power fluctuation which drastically affected the life span of the choke and starter component of the control gear systems. This was not cost efficient and greatly interfered with the 16 h/8 h light/dark regimen required for plant growth and morphogenesis. Its use has also been associated with high and expensive power consumption as well as a wide range of wavelength (350 -750 nm) that are unnecessary for plant development [1]. There was need to explore other lighting options for efficient running of a functional tissue culture laboratory in the country.

Recently the newly developed light-emitting diode (LED) has emerged as an alternative light source [1] and has a comparative advantage over FL for havingspecific wavelengths, small mass and volume, long useful life, low heating and efficient light generation process. They also do not contain mercury or other elements that might be hazardous to the environment [14, 7]. However, the cost associated with the initial installation of LED lights is beyond the scope of most laboratories in developing countries like Nigeria. The aim of this study was to evaluate the potential of compact fluorescent light (CFL) as alternative to FL and determine the effect of light intensity and orientation on *in-vitro* propagation of root and tuber crops. These are compact version of the FL but are of different sizes and shapes that enable it fit into the fixtures of incandescent bulbs. It has a compact electronic ballast or control gear in the base of the lamp which is not as affected by current fluctuations as with FL. Although wide spread illumination is obtained using FL, same effect could be achieved with CFL with a number of CFL arranged at different orientation. To optimize light spread with CFL we looked at the effect of three different arrangement or orientations; horizontal, angular and vertical on plant height, leaf and root number and the number of regenerated plantlets.

II. Materials And Method

2.1. Light source and arrangement:

CFL bulbs of wattage 5, 9, 11 and 40 (Fig.1) produced from different manufacturers were sourced from local marketin Umuahia, Abia State, Nigeria. The choice of bulbs was based on availability in local market and durability. The 5W and 9W were manufactured by Panasonic while 11W and 40W were produced by TORCH companies. Bulbs of the same intensity were arranged in parallel along racks of five shelves each and in series within shelves (Fig. 2). This arrangement was similar to what was obtainable with white fluorescent tubes in laboratoriesworldwide. Different light arrangements of CFL bulbs comprising of horizontal, angular, and vertical orientations (Fig. 3) were constructed to achieve the same level of light distribution produced by fluorescent tubes.Three CFL bulbs were installed within each shelf for the three different orientations.

For angular orientation, three CFL bulbs were arranged at an angle of 45ºC from each other to achieve uniform light distribution within the shelves (Fig 3B), while three vertical CFL bulbs were arranged side by side in series to achieve additive effect of the three bulbs concentrating light in a small space (Fig. 4C). The horizontal orientation has three CFL bulbs fixed in an angular lamp holder arranged side by side in a series. The experiments were set up in complete randomized design.

2.2. Plant establishment

Four weeks old *in-vitro* propagated cassava mother plants were sub-cultured in Murashige and Skoog basal medium (MS3) supplemented with 3% sucrose and MS vitamin. The medium was dispensed into three different culture vessels, 25 x 10 mm disposable plastic petri dishes, 25 x 150 mm pyrex test tubes and 32 oz (946 ml) baby food jar. The disposable petri dishes and baby food jar were inoculated with three cassava nodal cuttings each, while the test tube was inoculated with one cassava nodal cutting. The three culture vessels represented internal replicates of the experiment and mean of the data derived from these vessels were used in statistical analysis. The experiment was also replicated thrice along the culture racks. The cultures were incubated at 25 + 2ºC temperature at 16h / 8h photoperiod.

Effect of light intensity, orientation and interaction of both factors on plant height, leaf node formation, root proliferation and number of regenerated plants were determined and analyzed statistically.

Unit conversion

To convert the radiant flux incident on a receiving surface from all directions, per unit area of surface (irradiance) expressed as Wm^{-2} (PAR) to µMol m^{-2} s^{-1}, the value in watts was multiplied by 4.6 for white fluorescent light (www.Li-cor.com). The correspondingµMol $m^{-2}s^{-1}$ values for the light intensities used in this study are shown in Table 1.

Statistical analysis

A multifactorial ANOVA analysis was carried out using Genstat statistical software and mean separation carried out manually using Duncan's method.

Fig. 1: Different sizes and wattage of CFL bulbs used based on availability in the local market. (A) 5 watts bulb from Panasonic, (B) 9 watts bulb from Panasonic, (C) 11 watts bulb and (D) 40 watts bulb from Torch companies.

Table 1: Radiant energy conversion

S/n	Watts: (Wm^{-2})	Photon ($\mu Mol\ m^{-2}\ s^{-1}$)
1.	5	22.95
2.	9	41. 31
3.	11	50. 49
4.	40	183.6

Fig.2: Serial and parallel arrangement of different intensities of plant. Showing light arranged in series across shelves (horizontal) and in parallel along racks (Vertical). Each rack is controlled by a separate switch

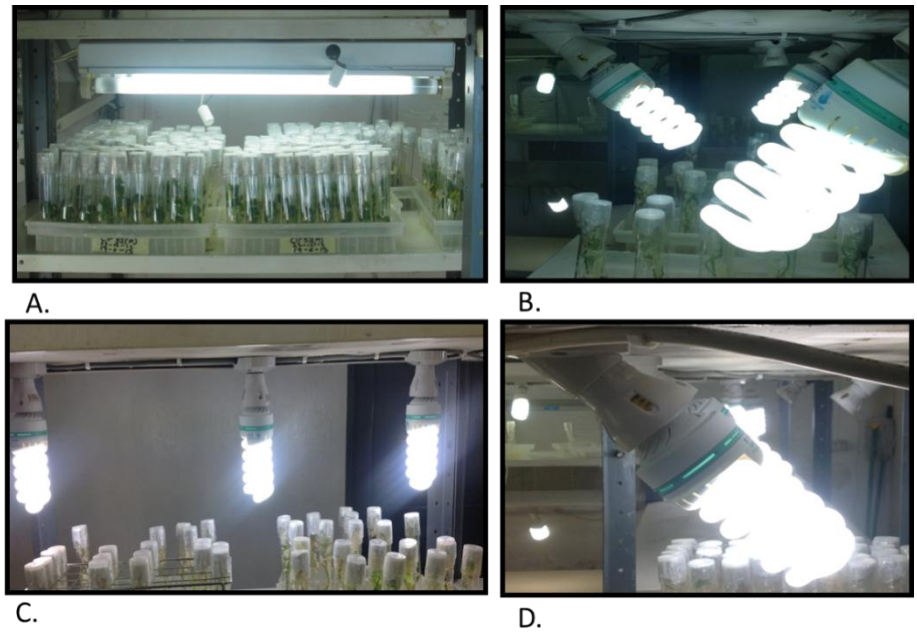

Fig. 3: Different Light arrangement/orientation to achieve uniform light spread in a given shelf area; (A) shows fluorescent tube arrangement giving a uniform light spread over a given shelf area. (B) Angular orientation with three CFL spiral bulbs arranged at different angles to achieve uniform light spread within the shelf (C) Vertical light orientation with CFL bulbs concentrating light in a smaller space whose effect is triplicated by using three CFL bulbs and (D) Horizontal light orientation with three CFL tube side by side at sample angle to achieve uniform light spread within the shelf

III. Result

The effect of light intensity on *in-vitro* regeneration of cassava plantlets is shown in Figure 4 and data obtained showed that the number of leaf nodes and roots formed did not vary across the different levels of light intensity. However, the number of regenerated plantlets and plant height obtained from 9 watts differed from those obtained from 5, 11 and 40 watts. Analysis of variance (Table 2) shows that the differences in the mean values of the numbers of leaf nodes and roots across the different levels of light intensity were not significant at $p = 0.246$ and 0.208 respectively. While the mean values of plant height and number of regenerated plants were significantly different at $p = 0.05$ and $p<0.001$ respectively. Post hoc analysis showed that 40 watts gave the highest value for plant height, followed by 11 watts, while the least value for plant height was derived from 9 watts (Table 2). Conversely, the highest number of regenerated plantlets was derived from 9 watts, followed by 11 watts with 5 watts producing the least number of regenerated plantlets.

The effect of light orientation on plant height and the number of leaf nodes, roots and regenerated cassava plantlets are shown in Figure 5. Data displayed showed that the number of leaf nodes formed was not affected by light orientation while plant height, root number and number of regenerated plantlets where affected by differences in light orientation. However ANOVA analysis showed that light orientation had no significant impact on plant height at $p = 0.572$ and number of leaf nodes at $p = 0.349$, while the effect on root proliferation and number of regenerated plantlets are highly significant at $p<0.001$ as shown in Table 2.Mean separation analysis for root proliferation and number of regenerated plantlets showed that horizontal orientation had the least impact on both root proliferation and plantlets regeneration while vertical and angular orientations had the most impact on root proliferation and plantlet regeneration, respectively. The interactive effect of light intensity and orientation on numbers of leaf nodes and roots formed were non-significant at $p = 0.06$ and 0.211 respectively but were highly significant on number of regenerated plantlets ($p<0.001$) and plant height ($p = 0.008$).

Results from the data analysis, showed that leaf node formation is least affected by light intensity and orientation and could not be changed by varying these factors. On the other hand, plant regeneration was significantly affected by light intensity, orientation and interactive effect of light intensity and orientation. Root proliferation was not significantly impacted by light intensity but varied significantly with light orientation while plant height was significantly increased at light intensity of 40 watts and by the interaction of light intensity and orientation.

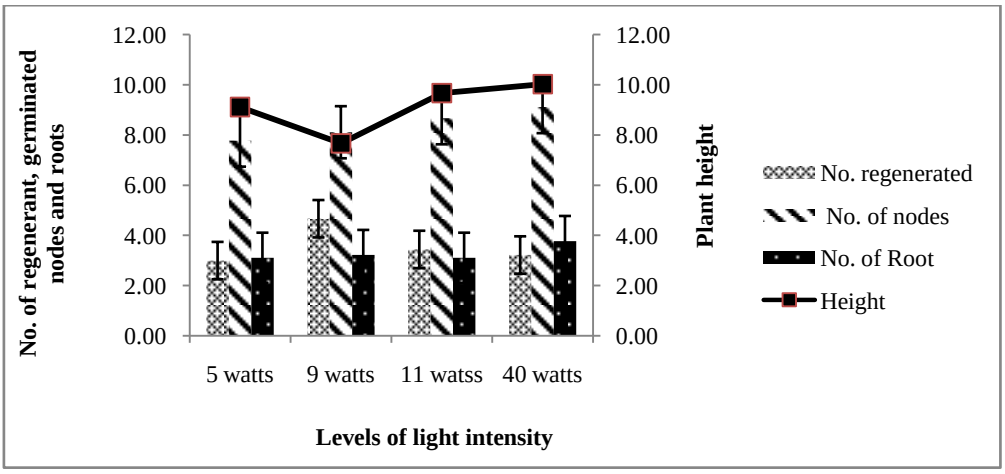

Fig.4: Effect of light intensity on *in-vitro* regeneration of cassava plantlets showing the response of plant height, number of leaf nodes, roots and regenerated plantlets to different light intensities

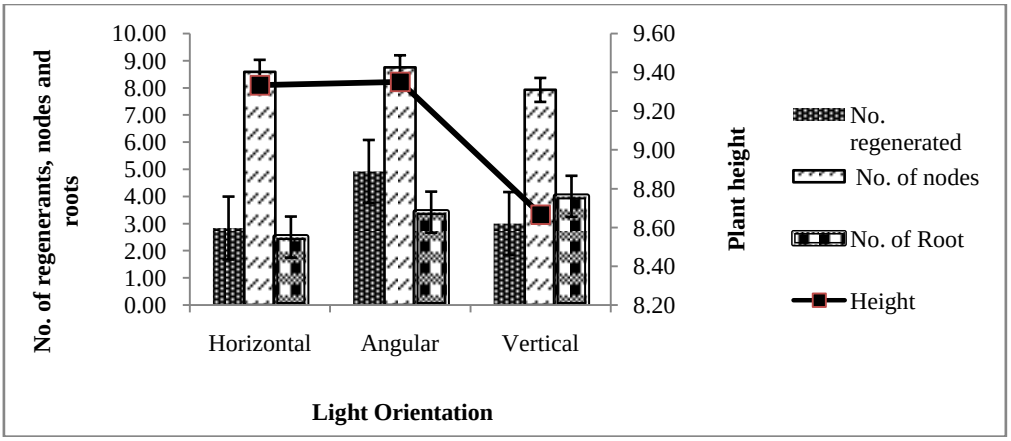

Fig.5: Effect of light orientation on *in-vitro* regeneration of cassava plantlets showing the response of plant height, number of leaf nodes, roots and regenerated plantlets to horizontal, vertical and angular orientation of light.

Table 2: ANOVA table showing difference between means

Treatment	Plant height	No. of Leaf nodes formed	No. of Roots formed	No. of regenerated plantlets
Orientation				
Horizontal	9.33	8.52	2.5^{c}	2.83^{c}
Vertical	8.69	7.72	4^{a}	3^{b}
Angular	9.35	8.75	3.42^{b}	4.92^{a}
FLSD	1.509	1.231	0.632	0.647
Levels				
5 watts	9.11^{c}	9.78	3.11	3^{d}
9 watts	7.63^{d}	8.11	3.22	4.67^{a}
11 watts	9.67^{b}	8.67	3.11	3.44^{b}
40 watts	10.02^{a}	9.11	3.78	3.22^{c}
FLSD	1.743	1.421	0.732	0.747
F-test				
Orientation	ns	ns	***	***
Level	*	ns	ns	***
Orientation x levels	**	ns	ns	***

IV. Discussion

This study was targeted at improving tissue culture systems in developing countries like Nigeria by improving the supply of light source required for efficient plant growth and morphogenesis. White fluorescent tubes (FL) mostly used in tissue culture laboratories were replaced with compact fluorescent bulbs (CFL). Although both light sources have the same basic principles and efficiency, they differ only in size and design.

The CFL bulbs are made in special shapes to fit into standard household light socket and also have integral ballast or control gear that is built into the light bulb. On the other hand, the FLs are long tubes requiring different light socket and separate ballast comprising of starter and choke independent of the bulb. The short life span of chokes and starters as a result of current fluctuation was the main constraint in the use of FL for tissue culture purposes.

Although most studies have shown that use of Light Emitting Diode (LED) has comparative advantage over the use of white fluorescent light in terms of specific wavelength, life span,low heating and efficient light generation [14, 7], the initial capital investment required for its initial installation makes this technology a far reach for most tissue culture laboratories in Nigeria. Use of CFL was adopted to mitigate these challenges; however there was the emerging challenge of determining the exact type (in terms of light intensity), number and arrangement of CFL that will be optimal for *in-vitro* plant regeneration.Factors considered as indices for plant regeneration include plant height, number of leaf nodes (particularly for cassava used in this study), number of roots formed which is essential for ex-vitro acclimatization and number of plantlets regenerated.

Data from this study have shown that light intensities in the range of 23 – 184 µmol m^{-2} s^{-1}(photons produced from 5W, 9W, 11W and 40W) generated from CFL and the horizontal, vertical and angular orientation of these bulbs have no significant impact on number of leaf nodes formed. Contrarily, plant height has been shown to be affected by light intensity and interaction between light intensity and orientation but not by orientation alone. It would have been expected that factors affecting plant height would have automatically affected the number of leaf nodes formed. However, from observation it has been noted that leaf nodes are not equally spaced on a particular plantlets and may not necessarily populate the whole length of a shoot. Sometimes, some plants can have very short internodes thereby suggesting that plant length and number of nodes formed are not often correlated. The findings of this study corresponds to the findings of Niedz*et al*. [10] showing that light intensity in the range of 20 to 89 µE which is equivalent to 20 to 89 µmol m^{-2} s^{-1} produced equivalent number of shoots/explants from citrus epicotyl explant. Node formation in cassava and in most other clonally propagated crops is equivalent to the number of shoots generated as the number of clones derived from single plantlets is determined by the number of nodes formed.

Post hoc analysis of the effect of light intensity on plant height showed that light intensity of PFD 184 µmol m^{-2} s^{-1} equivalent to the irradiance produced by 40 W CFL gave the highest value for plant height followed by PFD of b51 µmol m^{-2} s^{-1} (11 W). PFD of 184 µmol m^{-2} s^{-1} observed in this study seems to contradict the data recorded for other plant species. For *Alocassaiaamazonina,* Rajesh,*et al*. [16] showed that plant growth and morphological development were optimum at PFD of 30 µmol m^{-2} s^{-1} but decreased at higher light intensity of PFD of 60 or 90 µmol m^{-2} s^{-1}. Similarly, Buah*et al*. [11]reported that dwarf cavendish banana showed superior growth, multiplication and chlorophyll content at light intensities of 5000 lux (70 µmol m^{-2} s^{-1}) which declined significantly at light intensities of 4000 lux (56 µmol m^{-2} s^{-1}) and 6000 lux (84 µmol m^{-2} s^{-1}). Also reports from Soontornchainksaeng*et al*. [17] showed that light intensities of 74 µmol m^{-2} s^{-1} was optimal for dry matter accumulation, plant height, leaf number and leaf area development for *Phaiustankervillae* and *Vanda Coerulea*. This report shows that the wide range of light intensity between 51 to 184 µmol m^{-2} s^{-1} that favored plant height in cassava could accommodate the regeneration of other plantlets except for banana that seems to be affected at both ends of light intensity.

This study also showed that the number of plantlets regenerated from cassava nodal cuttings was affected by light intensity and orientation as well as interaction between light intensity and orientation. Mean separation of the means derived from the 4 light intensities used in this study showed that 9 watts (41 µmol m-2 s-1) gave the highest value for the number of regenerated plantlets followed by 11 watt (51 µmol m^{-2} s^{-1}). This result agrees with the findings of Sengari*et al*. [9] in their work with two sugarcane varietiesshowing that the maximum number of shoots derived per culture was obtained at 4000 lux equivalent to 53 µmol m^{-2} s^{-1}. Although optimum plant regeneration was obtained with nearly the same light intensities in both plants, the trend observed in sugarcane differed with that observed in cassava. For sugarcane, increase in light intensity increases the regeneration frequency and number of shoots derived per culture, whereas in our case, the PFD of 184 µmol m^{-2} s^{-1} equivalent to 40 W gave lower values for number of regenerated plantlets comparedto 11 W (51 µmol m^{-2} s^{-1}) and 9 W(41 µmol m^{-2} s^{-1}). This may be attributed to differences in crop types as well as difference in bulb manufacture. Bulbs available at the time of study in the locality where the research was carried out was used in order to facilitate the adoption of the recommendations of the study. Unfortunately, the capacity of the bulbs used in this study was not all provided by the same manufacturer and perhaps might be interesting to compare the efficiency of bulbs provided by many manufacturers.

Root proliferation on the other hand, is shown to be affected by light orientation only and not by light intensity. Vertical orientation had the most impact on the number of roots formed followed by angular orientation. Similarly, for plant regeneration; the highest value was produced by angular orientation and since white fluorescent tubes and lately LED tubes are mainly employed as *in-vitro* light sources, this study provides the very first data on effect of light orientation as it relates to CFL on *in-vitro* propagation of plants.

V. Conclusion

Based on the results obtained from this study and the various factors affecting the four indices; leaf node formation, plant height, plantlet regeneration and root proliferation, light intensity of PFD 51 µmol m^{-2} $s^{-1}$and angular orientation was chosen as optimal for *in-vitro* propagation of cassava. The PFD of 51 µmol m^{-2} s^{-1} generated from 11W bulb being the second best for plant regeneration and plant height was chosen since the 9W (41 µmol m-2 s-1) and 40W (184 µmol m^{-2} s^{-1}) bulbs optimal for plantlet regeneration and plant height respectively were not optimal vice versa. Also, angular orientation that had the best impact on plant regeneration and second best on root proliferation was chosen over vertical and horizontal orientations. Vertical orientation favored root proliferation only while horizontal orientation had the least impact on all the four indices. Results obtained in this study are essential towards effective operation of tissue culture laboratories in Nigeria and other developing countries.

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