MICRO PROPAGATION OF *Pterocarpussantalinoides* USING THREE DIFFERENT GROWTH MEDIA

ABSTRACT

Aims: Micro propagation of *P. santalinoides* was carried out in order to ascertain the most appropriate culture media for its micro propagation
Study Design: The experiment was laid out in different growth media in the laboratory.
Place and Duration of Study: The micro propagation of *Pterocarpussantalinoides* was carried out at the Tissue culture laboratory of the University of Nigeria, Nsukka and lasted between July and October 2018.

Methodology: Seeds from fresh and healthy ripe fruit which was cut open mechanically with the help of secateurs was gotten from Ai-kwu,Otukpa Local Government Area of Benue State, Nigeria. The seeds were air dried and used as explant. The explants were surface sterilized using NaOCl solution for 10mins, rinsed with distilled water and then the soft seed coat were removed and the seeds were cultured under aseptic conditions on MS medium and other growth medium. Seeds of *Pterocarpussantalinoides* were inoculated on six different growth media with varying compositions. The media are MS, B5 and white's without growth hormones (MSoo, B5oo, and WHoo), and each of them was supplemented with 3.0mgl^{-1} BAP and 0.5 mgl^{-1} NAA (MSBN, B5BN, WHBN).

Results: Seed germination improved in all the media studied. However, MS combinations gave the best result (90-93%). The maximum number of leaves and roots recorded was in MSBN (3.8 for leaves and 2 for roots) followed by MSoo (2.6) and WHBN (2.6). The leaf area was best for the MS combination (0.232cm^2) followed by the White's combinations (0.154cm^2) and least for the B5 combinations (0.026cm^2) while shoot and root length was maximum in MSBN (4.28cm for the shoot and 1.18cm for the root) followed by WHBN (1.90cm).

Conclusion: The growth rates of *Pterocarpussantalinoides*, in MS medium among other basal media (B5 and White) offers a compromise between all the growth parameters which indicates that variation of the basal medium composition could lead to enhanced *Pterocarpussantalinoides* regeneration efficiency.

Keywords: Micropropagation, growth medium, Pterocarpussantalinoides, Inoculated, Shoot, Roots

1. INTRODUCTION

The plant *Pterocarpussantalinoides* (L'Herit ex Dc) is a dicotyledonous plant belonging to the family Fabaceae. It is a tree that grows along riverine forests of Africa and tropical South America and is a native of Brazil, Cameroon, Ghana, Nigeria, and Senegal [11]. The plant flowers from December to March and fruit ripening is between March and April. The Nigerian species are trees with light yellow flowers and they usually have alternate leaflets [11].
The natural resurgence of *Pterocarpussantalinoides*isthrough seeds. However their germination rate has been reported to be very poor which is attributed in part to impervious seed coat and its propagation through stem cuttings poses difficulties and also, over exploitation is putting further pressure on the species which is at the verge of extinction if proper steps are not taken for it conservation. In view of the problems of conventional propagation and lack of sufficient information on the *in vitro* propagation and response of various culture media, it was therefore necessary to carry out this study.
In Nigeria the plant is usually found in the Eastern, Southern and Northern parts. The vernacular names include Hausa(gunduru); Igbo(nturukpa); Idoma(atukpa) and Yoruba(gbengbe).
In Nigeria, various partsof the plant are used in traditional medicine to treat an array of human ailments. The fresh leaves of the plantare consumed locally in soups by the Igbos of South East Nigeria and are reputed to be useful in the treatment of diarrhoea and other gastrointestinal disorders [3]. Also, the fresh-

leaf extract combined with leaves of *Solanummacrocarpum*is used in the management of high blood pressure with similar uses reported among the Igede tribe in Benue State, Nigeria [7]. The leaves of the plant are also eaten as vegetable serving as food for man, livestock browse its young shoot as fodder, the wood is white or yellow,not hard but termite-resistant and used for construction purposes,the bark contains tannins and dyes for dyeing and it is also medicinal because the tree bark is used as a stomach ache remedy [10]. The plant also provides useful services such as erosion control because it is an important species for soil conservation in water catchment areas, a good windbreak around settled areas and farms; it also forms nodules with nitrogenase activity thereby helping in nitrogen fixation. Leaf litters from the plant decompose slowly to release nitrogen and significantly increases soil exchangeable Calcium and Magnesium and hence improve soil fertility. It is also a beautiful tree with good gardening attributes [1]. In south-east Nigeria, the leaves of the plantis ethnomedicinally used against gastrointestinal diseases, diabetic syndrome and is known to exhibit antipyretic property [2]. The leaves are also used for the treatment of skin diseases [4]. Its stem-bark and leaf extracts are said to possess anti-enteropooling, antimalarial, anti-abortive and antibacterial properties [9], [2]. The use of the leaves in veterinary medicine[4],fodder for livestock [11], and together with leaves of *Solanummicrocarpum* in management of high blood pressure [3] and many others have been reported.

Based on it medicinal use, it is used among the Idoma and the Igede people of North Central Nigeria in the treatment of inflammation of lower abdomen/lower abdominal pain, stomach ache and other infectious diseases [6].

Micro propagation is a technique that manipulates small quantities of axenic plant materials ranging from single cells to stem segments, under conditions favorable to the formation of newplants. It has proven to be the most efficient and cost-effective method of propagating large numbers of clonal offspring for many agronomic crops, including both herbaceous and woody perennial species.

Older and simpler techniques of cloning plants (cuttings, grafting, and division of parent stock material) are limited by seasonal constraints and the natural formation of new plant structures. Micropropagation on the other hand, allows the year-round production of new plants at rates significantly higher than those achievable by all other methods. The plants produced are genetically uniform, vigorous, and free from associations with other organisms

2. MATERIALS AND METHODS

2.1 Place of Study

The research work was carried out in the Plant Tissue Culture Laboratory of the University of Nigeria, Nsukka.

2.2 Collection of Explants (Seeds)

Seeds from fresh and healthy ripe fruits which were cut open mechanically with the help of secateurs were gotten from Ai-kwu in Otukpa Local Government Area of Benue State, Nigeria and where air dried. The seeds were subjected to viability test. They were soaked in water for 15-20 minutes after which the seeds that floated were considered non-viable and hence, they were discarded while the seeds of uniform size that sank were used for the study.

2.3 Preparation of Explant

These seeds soaked in sterilized water were kept overnight and used for culture on the following day. Before culturing, they were surface sterilized using NaOCl solutions for 10mins, followed by rinsing with distilled water. Soft seed coats were removed and the seeds were cultured under aseptic conditions on MS medium [8] without growth hormones. Seed cultures were incubated separately. After which germinated plantlets with nodes were observed for 35-40 days.

2.4 Nodal Segment Culture

The nodal segment from germinated plantlets was used for initial culture. The nodal segment with a single node was excised under aseptic conditions and used for culture media.

2.5 Culture Media and Condition

Three basal media namely MS, B5 [5] and White's (WH) (White, 1963; Cultivation of Animal and Plant cells) were used for the initial culture of the nodal segments. Each of them without growth hormones was used as control. All the culture media were supplemented with sucrose and agar and the pH was adjusted. The media was autoclaved at the appropriate temperature for 20minutes and culture tubes containing one nodal segment each were incubated.

2.6 Inoculation

2.6.1 Preparation of Inoculation Chamber

The inoculation chamber (Laminar air –flow cabinet) was properly washed cleaned and allowed to dry. The surfaces were swabbed with absolute alcohol (ethanol) and ultra violet light was switched on for about 30 minutes to sterilize the environment after which all the needed material for the inoculation were kept inside the inoculation chamber and the laminar air flow was switched on.

2.6.2 Inoculation Procedure

The seeds of *Pterocarpussantalinoides* were carefully selected and pre sterilized by a quick dip for few seconds in 70% ethanol. It was followed by sterilization which was by submerging the explants into 10% sodium hypochlorite (NaOCl) for 25 minutes; the sterilizer used was commercial bleach (Jik). After which the seeds were rinsed with 3 changes of sterile distilled water to remove residues of the sterilizer from the seeds.With the aid of forceps, the sterile seeds were transferred into well labeled culture tubes placed in culture racks and kept in a growth room. Twenty test tubes for each treatment were placed in growth room. The date of the inoculation was noted and germination of the explants (seeds) was monitored daily. Germination readings were taken until there was no further seed germination. Also germination rate was noted under each treatment.

2.7 Germination Studies

The cultured seeds were regularly monitored as they germinated over a period of 30 days starting from the day of inoculation.

2.7.1 Percentage Germination

The seeds that germinated daily under each treatment were counted and expressed as a percentage of the total number of seeds planted. The formula below was used for the calculation

$$\text{Percentage germination} = \frac{\text{Number of germinated seeds} \times 100}{\text{Total number of seeds planted}}$$

2.7.2 Germination Rate

This is the reciprocal of the day or time 50% germination is attained. The formula is thus

$$\text{Germination rate} = \frac{\text{Number of germinated seeds} \times 100}{\text{The day or time 50\% germination is attained}}$$

2.8 Determination of Other Growth Parameters

Seedlings from each treatment at the end of the experiment were sampled destructively to obtain the following parameters.

2.8.1 Number of Leaves

The number of leaves was visually counted and recorded.

2.8.2 Leaf Area

The leaf area of each seedling was determined by paper tracing method, the leaf was detached and placed on a graph sheets and the outline traced. The number of boxes covered was added to the value obtained by dividing the number of uncovered boxes by 25 which is the number of boxes of each square of the graph sheet.

2.8.3 Length of Shoot

The length of the shoot was taken with the aid of a long thread which was later superimposed on a meter rule and the measurement taken in cm.

2.8.4 Number of Roots

The number of roots were visually counted and recorded.

2.8.5 Length of Roots

The length of the root was measured with the aid of a long thread which was later superimposed on a meter rule and the measurement taken in cm.The data on the number of shoots per explants, number of nodes per shoot and shoot length were recorded after 45 days of inoculation of the nodal explants and were subjected to analysis using CompleteRandomized Design.

3 RESULTS AND DISCUSSION

3.1 Percentage Growth Rate

The percentage growth rate for all the growth media used was recorded. (Table 1) the table revealed that the percentage growth rate was highest in the MS medium with growth hormone (93%), followed by MS medium without growth hormone with 90% growth rate, White medium with growth hormone with 78% germination rate, White medium without growth hormone with 75% growth rate, B5 medium with growth hormone of 66% growth rate and the least was recorded on the B5 medium without growth hormone with 60% growth rate. This information is also represented with a pie chart (figure 1)

Table 1: Percentage growth rate

Growth Media	Percentage Germination Rate
MS medium without growth hormone	90
MS medium with growth hormone	93

B5 medium without growth hormone	60
B5 medium with growth hormone	66
White medium without growth hormone	75
White medium with growth hormone	78

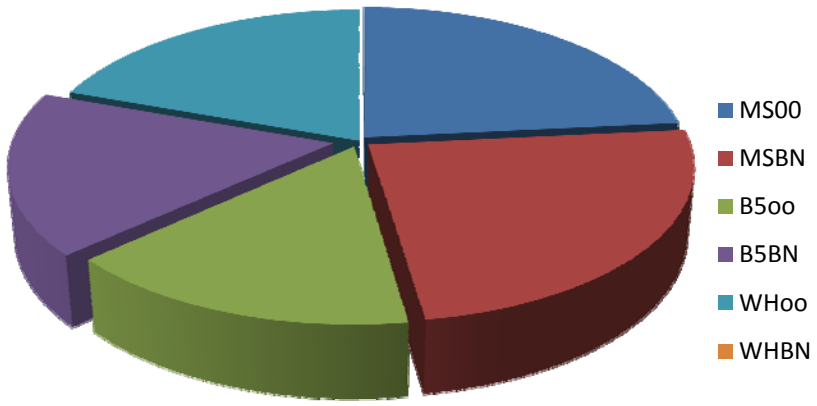


Figure 1: Pie chart showing percentage growth rate

Key:

MS oo – MS medium without growth hormone, MSBN – MS medium with growth hormone

B5oo – B5 medium without growth hormone, B5BN – B5 medium with growth hormone

WH oo – White medium without growth hormone, WHBN – White medium with growth hormone

3.2 Number of Leaves and Shoot

The difference between the number of leaves and number of roots using different growth media is shown in Table 2. The table shows that the MS medium with growth hormone has a mean value of 3.8 for leaves and 2 for roots, MS medium without growth hormone with a mean value of 2.6 for leaves and 1 for roots which is the same number for White medium with growth hormone, B5 medium with growth hormone has a mean value of 2 for number of leaves and 0.2 for number of roots, White medium without growth hormone with mean value of 1.6 for leaves and 0.816 for the number of roots and the least was recorded for B5 medium without growth hormone with a mean value of 1 for the number of leaves and 0.1 for the number of roots. This information is represented with a bar chart (Figure 2)

Table 2: Mean Values for Number of leaves and roots

Growth Media	Number of Leaves	Number of Roots
MS medium without growth hormone	2.6	1
MS medium with growth hormone	3.8	2
B5 medium without growth hormone	1	0.1
B5 medium with growth hormone	2	0.2
White medium without growth hormone	1.6	0.816
White medium with growth hormone	2.6	1

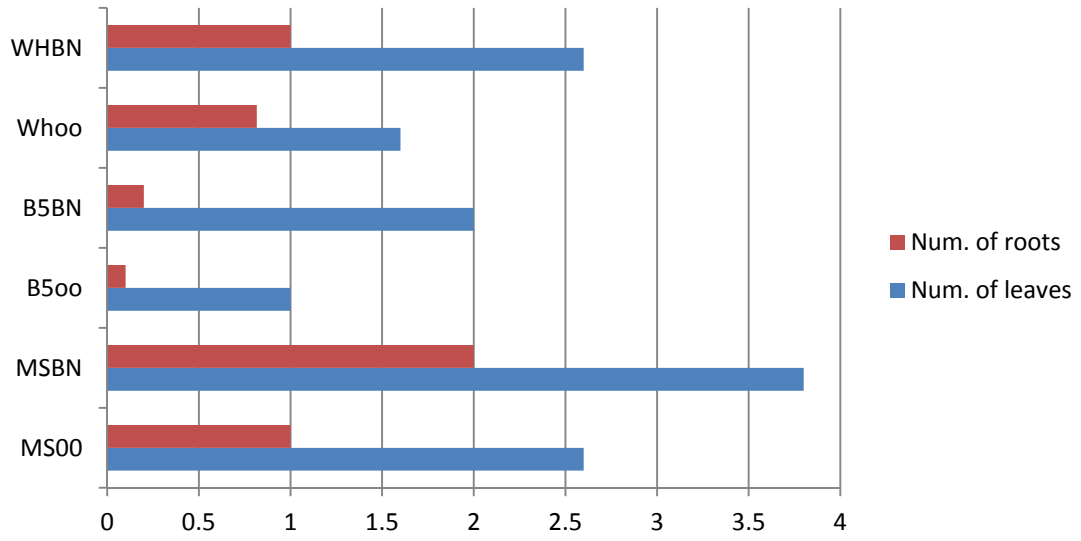


Figure 2: Bar chart showing Mean Values for number of leaves and roots

Key:

MS oo – MS medium without growth hormone, MSBN – MS medium with growth hormone

B5oo – B5 medium without growth hormone, B5BN – B5 medium with growth hormone

WH oo – White medium without growth hormone, WHBN – White medium with growth hormone

3.3 Leaf Area for the different growth media

The mean value for the leaf area using different growth media is shown in table 3 and figure 3. MS medium with growth hormone has the highest leaf area with 0.232 cm^2, White medium with growth hormone (0.154 cm^2), MS medium without growth hormone (0.077 cm^2), White medium without growth hormone (0.04 cm^2), B5 medium with growth hormone (0.026 cm^2) and the least was recorded for B5 medium without growth hormone (0.021 cm^2)

Table 3: Leaf Area

Growth media	Leaf Area (cm^2)
MS medium without growth hormone	0.077
MS medium with growth hormone	0.232
B5 medium without growth hormone	0.021
B5 medium with growth hormone	0.026
White medium without growth hormone	0.04
White medium with growth hormone	0.154

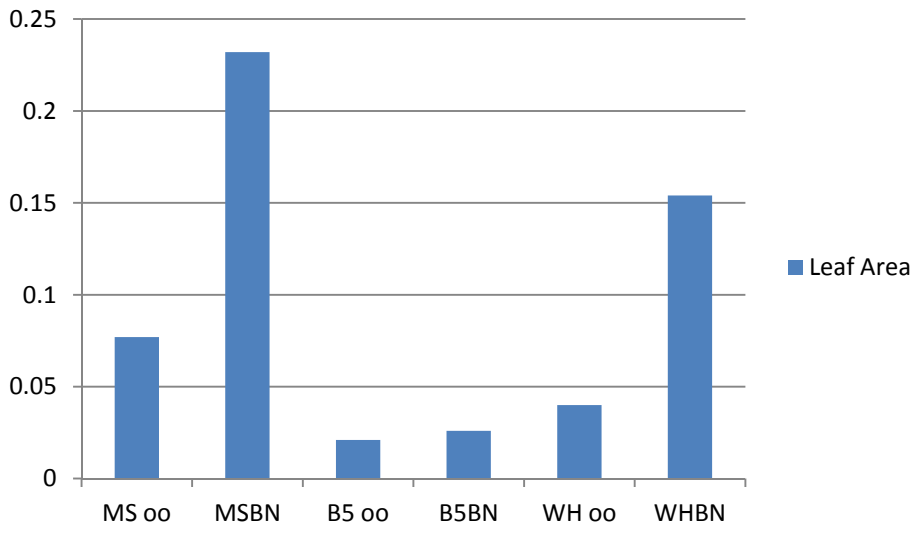


Figure 3: Column chart showing mean values of leave area

Key:

MS oo – MS medium without growth hormone, MSBN – MS medium with growth hormone

B5oo – B5 medium without growth hormone, B5BN – B5 medium with growth hormone

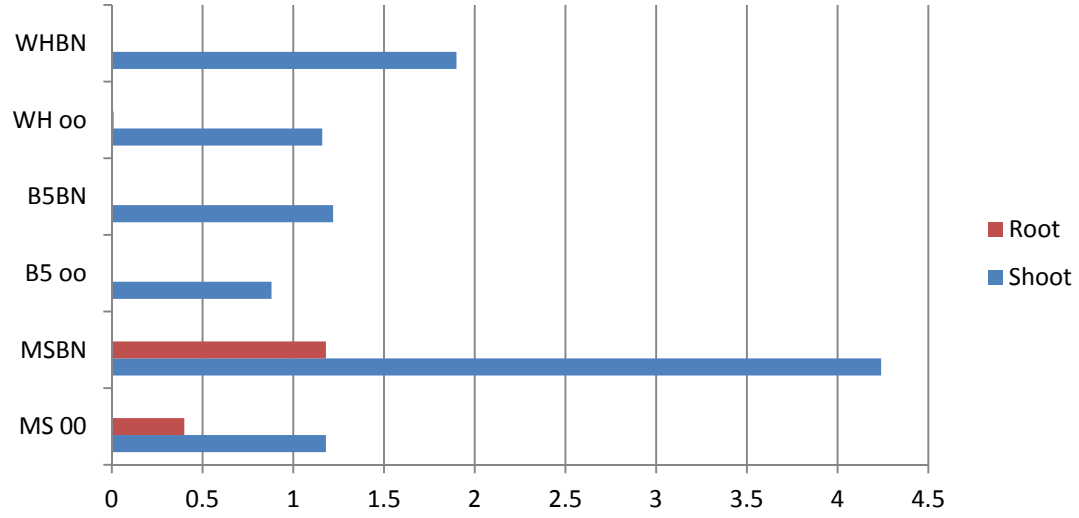
WH oo – White medium without growth hormone, WHBN – White medium with growth hormone

3.4 Mean values for length of shoot and roots

The mean values for length of shoot and roots on different growth media is shown in table 4 and figure 4. The table revealed that MS medium without growth hormone has a value of 1.18 for shoots and 0.4 for roots, MS medium with growth hormone has 4.24 for shoot and 1.18 for roots, B5 medium without growth hormone has a value of 0.88 for shoot and 0 for roots, B5 medium with growth hormone has a value of 1.22 for shoot and 0 for roots, White medium without growth hormone has a value of 1.16 for shoot and 0.01 for roots while White medium with growth hormone has a value of 1.9 for shoot and 0.2 for roots.

Table 4: Mean values for length of shoot and roots on different growth media

Growth Media	Shoot (cm)	Root (cm)
MS medium without growth hormone	1.18	0.4
MS medium with growth hormone	4.24	1.18
B5 medium without growth hormone	0.88	0
B5 medium with growth hormone	1.22	0
White medium without growth hormone	1.16	0.01
White medium with growth hormone	1.9	0.2



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Key:

MS oo – MS medium without growth hormone, MSBN – MS medium with growth hormone

B5oo – B5 medium without growth hormone, B5BN – B5 medium with growth hormone

WH oo – White medium without growth hormone, WHBN – White medium with growth hormone

A significant effect of the various culture media on shoot, leaf and root formation was observed in the present research. In line with the results obtained in this study,[12], who used MS medium in the propagation of *Menthaarvensis*reported an increase in rooting of regenerates when compared to other basal media. On the other hand, [14] also observed that a reduction in strength of MS medium resulted in the increase of *in vitro* shoot and root formation of high bush blueberry. [15] Conducted a similar experiment with this study on ginger to determine the effects of different media and there strength (full and Half MS strength), types (B5 and white's media) and source of nitrogen (NH_4NO_3) on shoot (number and length), leaf (number and area) and root number of ginger. His results were similar to those obtained in this study and indicated an increase of root length in MS basal medium when compared to other treatments. Though on a contrary, [13] reported that B5 basal medium proved to be superior to MS basal medium in callus-mediated regeneration from epicotyl and cotyledonary tissues of castor plant.

In this study, three basal media were compared (MS, White and B5) and all of them supported the *in vitro* regeneration of *Pterocarpussantalinoides*seeds, but MS medium was found to be significantly superior to both B5 and White media in all the growth parameters studied (length of roots, length of shoots, number of leaves and number of roots). These findings suggest that MS is better than both B5 and White for the seed germination *in vitro* of *Pterocarpussantalinoides*.

4. CONCLUSION

The growth rates of *Pterocarpussantalinoides*, in MS medium among other basal media (B5 and White) offers a compromise between all the growth parameters which indicates that variation of the basal medium composition could lead to enhanced *Pterocarpussantalinoides* regeneration efficiency.

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