Journal of Forestry Research and Management. Vol. 14 (2), 181-188; 2017, ISSN 0189-8418

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Growth Responses of *Nauclea diderrichii* (De Wild. and T. Durand) Merrill to Different Concentrations of Plant Growth Regulators Propagated through Seed Culture Technique.

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ABSTRACT

Nauclea diderrichii is among the list of vulnerable and threatened species due to over-exploitation and neglect as a result of the introduction of exotic tree species, therefore the need to determine the most appropriate protocol for its proliferation becomes imperative. Seeds of *Nauclea diderrichii* were propagated *in-vitro* to determine their growth responses to various concentrations of plant growth regulators. Six treatments were used in this experiment and there were twenty replicates per treatment. These were randomly arranged in the growth-room at 20 ± 2^{o}C under a 16 hours photoperiod for twelve weeks. After ten weeks, the growth response was determined by the evaluation of the shoot length, root length, total length, number of shoots and number of roots. Treatment F, which had 0.5mg/L Benzyl amino purine (BAP) and 0.1mg/L Naphthalene acetic acid (NAA) gave the longest shoot length of 11.0cm and also the highest average number of shoots per culture tube. This treatment proved that the higher the ratio of BAP to NAA, the greater the effect on the length of the shoot and the average number of shoots produced per tube. These results showed that the most adequate culture medium for obtaining the longest average root length (4.36cm) per culture after ten weeks was MS-medium supplemented with BAP at 0.2 mg/L and NAA at 0.1 mg/L, while the shortest average root length (3.34 cm) was exhibited by MS-medium supplemented with 0.5 mg/L (BAP) and 0.1 mg/L (NAA), this indicates that increasing the level of auxin (NAA) increases the length of roots and vice-versa.

Keywords: *Nauclea diderrichii*, concentrations, Naphthalene acetic acid, Benzyl amino purine, response.

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Introduction

Nauclea diderrichii (De Wild. And T. Durand) Merrill is a member of the Rubiaceae family. It is found in Angola, Cameroon, Central African Republic, and Republic of the Congo, Democratic Republic of Congo, Ivory Coast, Gabon, Ghana, Liberia, Mozambique, Nigeria, Sierra Leone, and Uganda. *N. diderrichii* was one of the dominant plantations in Nigeria (FAO, 1981), but the advent of large scale plantations with exotic species led to the situation where *N. diderrichii* was neglected (Onyekwelu *et al.*, 2003).

It was one of the 22 species identified in the early stage of forestry practice in Nigeria as a tree species of economic importance (Redhead 1971), thus it has been under intense exploitation from the end of the 19th century till date. Today, it is rare in the natural forests. Its exploitation prohibited in some states in Nigeria (FORMECU, 1999). Due to heavy exploitation, it is on the red list of the International Union of Conservation of Nature and Natural Resources (IUCN) as a vulnerable and threatened species hence the need for germplasm conservation to preserve it from extinction.

Although its natural regeneration has been described as very good and easy (Dupuy and Mille, 1993), regeneration under the natural tropical forest has not been successful, due partly to the complexity of the tropical forests and the multiplicity of species in it. Attempts to regenerate *N. diderrichii* systematically in the natural forest were completely given up in the 1960s and attention has subsequently shifted to regeneration through artificial means (FAO, 1981; Onyekwlu *et al.*, 2003), the need to determine the best or most suitable means of propagation *in vitro* is therefore imperative. This study sought to determine the responses of the seeds of *N. diderrichii* to various concentrations of plant growth regulators and determine the best protocol for the proliferation of *N. diderrichii* for the purpose of germplasm conservation and plantation establishment.

MATERIALS AND METHODS

Viable Seeds of *N. diderrichii* were collected from the Genebank of the Seed Store Section of Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State. The seeds were surface sterilized using 70% alcohol for 5 minutes and rinsed with sterile distilled water to remove the effect on the seeds. This was then followed by adding 10% Hypochloride for 15 minutes followed by 3 rinses in sterile distilled water. The surface-sterilized seeds were then cultured on Murashige and Skoog (1962) Basal medium supplemented with 3% w/v sucrose, 0.1g inositol and gelled with 0.7% w/v agar at various concentrations of cytokinin and auxin in 17ml test tubes. The cytokinin used was Benzyl-amino-purine (BAP), while the auxin used was Naphthalene acetic acid (NAA). All plant growth regulators were added before autoclaving and pH was adjusted to 5.7 ± 0.2 and autoclaved at 121°C for 15 minutes. The cultures were incubated in the growth room at 20 ± 2°C under a 16 hours photoperiod with

cool-white fluorescent light (Okere and Adegeye, 2011). There were six treatments, and twenty explants were cultured per treatment and later arranged randomly on the shelves in the growth room.

After ten weeks, the cultures were evaluated for shoot length, root length, total length, number of shoots and number of roots. The experimental layout was a completely randomized and the data obtained were subjected to analysis of variance. The observed means of the characters were subjected to Least Significant Difference (LSD) to show the mean separation. The various treatments of the media are shown in Table 1

Table 1: Different Media Concentrations.

	Treatments	BAP (mg/L)	NAA (mg/L)
1.	A (Control)	0.0	0.0
2.	B	2.0	1.0
3.	C	3.0	1.0
4.	D	3.0	3.0
5.	E	4.0	2.0
6.	F	5.0	1.0

RESULTS AND DISCUSSION

The longest shoot length (11.0cm) and highest average number of shoots (8) produced per culture were exhibited by explants cultured on the MS medium supplemented with 0.5mg/L BAP + 0.1mg/L NAA (Treatment F). Treatment F gave the greatest average shoot length indicating that the higher the ratio of BAP to NAA, the greater the effect on the length of the shoots and average number of shoots produced per culture (Table 2). This is in line with the study carried out by Okere and Adegeye (2011) on *K. grandifoliola.*

Table 2: Observable Effects of Treatments on the Seeds after 10 Weeks

Treatments	Longest shoot (cm)	Longest root (cm)	Longest plantlet (cm)	Average num of shoots (cm)	Average num of roots (cm)	Average shoot length (cm)	Average root length (cm)	Average plantlet length (cm)
A	6.8	6.9	11.4	1.25	4.35	3.98	4.01	7.99
B	9.5	5.5	12.3	2.7	4.15	4.88	4.36	9.24
C	6.8	4.9	11.2	3.7	8.2	4.84	3.56	8.40
D	9.3	4.7	13.1	3.25	4.45	6.26	3.68	9.94
E	7.5	5.2	11.3	4.85	7.3	5.12	3.80	8.91
F	11.0	8	12.5	8	5.2	6.38	3.34	9.72

These results showed that the most adequate culture medium for obtaining the longest average root length (4.36cm) per culture after ten weeks was MS-medium supplemented with BAP at 0.2 mg/L + NAA at 0.1 mg/L(Treatment B), while the shortest average root length (3.34 cm) was exhibited by MS-medium supplemented with 0.5 mg/L (BAP) + 0.1 mg/L (NAA),(Trt?) this indicates that increasing the level of auxin (NAA) increases the length of roots and vice-versa. These findings are in agreement with those reported by Kopadakova *et al.*, (2009), while trying to determine morphogenetic response to plant growth regulators in transformed and untransformed *Hypericum perforatum* L. clones. Treatment D (Plate 1) had the highest average shoot length.

Treatment E (Plate 2), which had high concentrations of BAP (0.4mg/L) + NAA (0.2mg/L) produced high average number of roots per culture in line with the works by Bustamante and Heras (1990) on *Nealolydia lophophoroides*; Feng-Feng *et al.*, (2000) on *Aloe barbebsis* and Mata-Rosas *et al.*, (2001) on *Turbinicapus laui* that using a high concentration of BAP and NAA in different concentrations will act as a limiting factor for shoot formation and also increases root formation.

Treatment C, which had 0.2mg/L BAP + 0.1mg/L NAA had the highest number of roots produced as against the average number of shoots produced. This could be due to the low concentration of BAP. It has been established that auxins like NAA can increase root formation in the presence of low cytokinins (Youssef, 1994).

Data in Tables 3 reveal that the different concentrations of the cytokinin (BAP) and auxin (NAA) used in this study had a significant effect on the proliferation of the plantlets of *N. diderrichii*.

Table 3: Mean shoot length, root length, total length, number of shoots and number of roots per culture.

BAP/NAA (mg/L)	shoots	roots	height	Depth	length
0/0 (Control)	1.25±0.55^{a}	4.35±2.62^{a}	3.98±1.46^{a}	4.01±1.32^{ab}	7.99±2.47^{a}
0.2/0.1	2.7±1.03^{b}	4.15±2.00^{a}	4.88±1.94^{a}	4.36±1.17^{b}	9.24±2.73^{ab}
0.3/0.1	3.7±1.53^{c}	8.2±3.38^{b}	4.835±1.73^{a}	3.56±1.05^{ab}	8.395±2.55^{ab}
0.3/0.3	3.25±1.33^{bc}	4.45±2.04^{a}	6.26±2.20^{b}	3.68±0.96^{ab}	9.94±2.77^{b}
0.4/0.2	4.85±1.76^{d}	7.3±3.83^{b}	5.115±1.71^{a}	3.795±1.00^{ab}	8.91±2.46^{ab}
0.5/0.1	8.0±1.84^{e}	8.0±1.84^{b}	6.38±1.35^{b}	3.34±1.30^{a}	9.72±2.08^{ab}

For each column means with the same superscript alphabets are not significantly different at 5% probability level

Plate 1: Multiple shoots per culture in Treatment D

Plate 2: Multiple shoots per culture in Treatment E.

CONCLUSION

In a report on studies on *in vitro* propagation of *Jatropha curcas* by Lapitan (2008) using different modified MS media with varying concentrations and combinations of different growth regulators, they were effective for different developmental changes in *Jatopha* tissues cultured. The media concentrations with more cytokinins in her experiment induced more shoot formation than the rest of the media tested as was the case also in this study. It can therefore be concluded that the regenerative capacity of seeds of *N. diderrichii* in some of the treatments used in this experiment can provide a great opportunity for the proliferation of the species for the purpose of germplasm conservation and future plantation establishment.

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