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IN VITRO MICROPROPAGATION OF *NAUCLEA DIDERRICHII* (DE WILD &T. DURAND) MERRILL: EFFECT OF NODES POSITION ON PLANTLETS GROWTH AND ROOTING

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Abstract

In tissue culture, the reactivity of explants in culture depends on their position on mother –plant or their physiological development level. This study aims to determine the regenerative potentialities of nodes according to their position for suitable *in vitro* micropropagation of *N. diderrichii*'s seedlings. Thus the effect of uninodal explants position of *Nauclea diderrichii* on seedling growth and rooting was studied *in vitro*. Three types of nodes (apical, middle and basal node) excised from two months old seedlings were tested using Woody Plant Medium (WPM) containing 30 g/L of sucrose and solidified with agar-agar at 8 g/L. The mean number of roots and shoots per plant was scored as well as the shoots and roots length was measured after six weeks of culture. Apical nodes produced seedlings with highest number of roots (6.80 ± 2.44 roots / plant) followed by basal (5.70 ± 2.68 roots / plant) and middle nodes (4.50 ± 2.12 roots / plant). But middle and basal nodes produced the best number of shoots (1.90 ±0.31 shoots / plantlet) than that obtained with apical nodes (1.30 ±0.57 shoots / plantlet). Seedlings obtained from apical nodes expressed efficient growth (4.70 ± 1.70 cm) compared to the middle (2.18 ± 0.97 cm) and basal nodes (2.33 ± 1.08 cm). So, for a rapid *in vitro* production of *N. diderrichii*'s seedlings, apical nodes of *in vitro* plants are more suitable.

Keywords: Nodal position, rooting, growth, *N. diderrichii, in vitro* micropropagation

Introduction

Nauclea diderrichii is a forest species which can reach 35 - 40 m of height. Member of Rubiaceae family, this species is mainly found in african rain forest (Wagenfuhr, 2000). Because of its high resistant wood against the attacks of fungus (*Coriolus versicolor*), Lyctus, termites (*Reticulitermes santonensis*) and marine borers (Gérard et al., 1998; Soro et al., 2014), it is abusively used. In Togo, this overexploitation and lack of its reforestation leads to its disappearance, as a result this species is classified on the list of the rare species (UNEP, 2010). Its regeneration *in situ* is laborious because of the dormancy of its seeds (Hawthorne, 1995a). According to Onyekwelu et al. (2003), because of the risk of extinction of this species, its artificial regeneration becomes necessary. Therefore *in vitro* micropropagation technique was adopted to ensure a large-scale mass production (Pitekelabou et al., 2015). *In vitro* vegetative micropropagation is a technique allowing to regenerate, to multiply and to keep an endangered wild species (Chen et al., 2001). The success of this technique depends on several factors among which the age or the position of the uninodal segment on the main axis of mother-plant from the apex to the basal nodes. *In vitro* vegetative multiplication can be made with nodes independently of their position. However, for the same age, a node can give various reactions according to the species in culture. According to Bona et al. (2012), the various types of node of many species of the same genus *Lavandula*, should give different reactions and that tests should be made before choosing the best type of node for a better multiplication. According to Luz et al. (2007) and Carvalho et al. (2007), the basal node induces more roots than the other nodes respectively in *Hydrangea macrophylla* and *Baccharis trimera*. *In vitro* micropropagation of *Macadamia spp*. revealed that the apical nodes did not regenerate any shoots and all of them withered while the first and second nodes which follow those apical nodes formed many shoots (Gitonga et al., 2010). Other authors showed on the other hand that the basal nodes gave more shoots than the apical nodes in *Rosa canina* (Shirdel et al., 2013) and *Persea americana* (Zulfiqar et al., 2009). The middle nodes expressed better results than the basal and the apical nodes in *Glycyrrhiza glabra* (Yadav and Singh, 2012). Thus, it was established that the various parts of a plant exhibit different regenerative potentialities (Mudoi et al., 2014). The study carried out here aims to determine the regenerative potentialities of the nodes according to their position for a rapid *in vitro* production of *N. diderrichii*'s seedlings.

Material and methods

Plant material used is constituted by uninodal leafless fragment of *N. diderrichii* excised from various positions (apical-middle and basal) on the main axis of two months old *in vitro* plants which are obtained by *in vitro* culture of seeds (Figure 1). Culture is initiated on Woody Plant Medium (WPM) without phytohormone. This medium consisted of Lloyd's and McCown's WPM macroelements (1980), 100 mg/L of myo-inositol, Murashige and Skoog microelements and vitamins (1962). This basic medium is supplemented with 30 g/L of sucrose and solidified with agar-agar at 8 g/L. The pH has been adjusted between 5.6 and 5.7 with NaOH at 1N or HCl at 1N. This medium was then distributed in tubes of 20 x 150 mm and sterilized at 120°C in autoclave under 1 bar of pressure during 20 minutes. The various nodes are placed on the WPM medium prepared above. Tubes containing the different nodes are stored in a culture room with a photoperiod of 16 h at 27 ± 2°C and a light intensity of 120 $\mu Em^{-2}s^{-1}$. The light is supplied by fluorescent lamps.

Observations are performed during six weeks. At the end of every week, the number of roots, shoots and nodes is scored and the shoots and roots length are measured by transparency through the test tube. Each measure is made on a population of twenty individuals and has been repeated twice. Data were subjected to the variance analysis (one-way ANOVA) and means were classified in homogenous groups according to Newman and Keuls's range test ($\alpha = 0.05$) using Statistica version 10 (Statsoft Inc; Tulsa, USA: 2011) program.

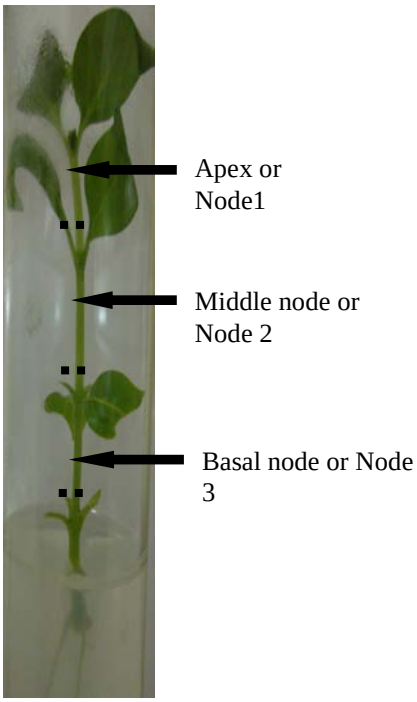

Figure 1: different types of nodes used for culture

Results

All the three type of nodes used as explants allowed root formation. The number of root initiated by the different type of nodes increased with time. Root formation was firstly observed with the apical nodes. After six weeks of culture, the mean number of root initiated by these explants was significantly higher than that obtained with middle and basal nodes. The roots initiated by the apical nodes, were significantly longer than those of the other type of nodes (table1).

Table 1: Mean number of roots and roots length of *N. diderrichii*'s seedlings produced by apical, middle and basal nodes on WPM

Types of nodes	Mean number of roots/plantlet	Length of roots /plantlet (cm)
Apical	$6.80^{b} \pm 2.44$	$4.50^{b} \pm 0.71$
Middle	$4.50^{a} \pm 2.12$	$2.45^{a} \pm 1.13$
Basal	$5.70^{ab} \pm 2.68$	$2.83^{a} \pm 1.30$

Average ± standard deviation of the measures made on 20 explants and repeated twice. The values affected by the same letter in the same column, are not significantly different according to the test of Newman-Keuls in $P < 0.05$.

The seedlings obtained from apical nodes exhibited a better growth compared to the others types of nodes (table 2). The shoots of these seedlings were bigger with longer internodes and larger leaves (Figure 2).

Table 2: Growth characteristic of *N. diderrichii*'s seedlings produced by different types of nodes on WPM

Types of nodes	Nodes/plantlet	Shoots/plantlet	Length of plantlet (cm)
Apical	3.40^{a} ±1.05	1.30^{b} ±0.57	4.70^{b} ±1.70
Middle	3.60^{a} ±0.82	1.95^{a} ±0.22	2.18^{a} ±0.97
Basal	3.60^{a} ±1.14	1.90^{a} ±0.31	2.33^{a} ±1.08

Average ± standard deviation of the measures made on 20 explants and repeated twice. The values affected by the same letter in the same column, are not significantly different according to the test of Newman-Keuls in $P < 0.05$.

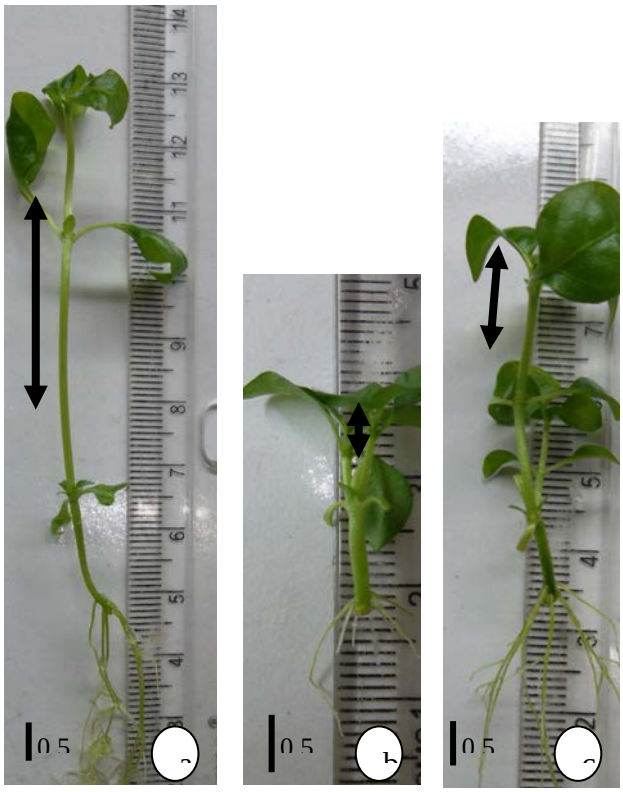

Figure 2: seedlings developed from apical (a), middle (b) and basal nodes (c) after six weeks culture

= Length of the internodes

Discussion

The development of *in vitro* seedlings not only depends on the nature of the explants, but also on its stage of development. In this study, apical nodes expressed better responses than the other nodes. Indeed, shoots induced by apical nodes elongated and rooted more efficiently than the other two types of nodes. This result is similar to that obtained by (Zulfiqar et al., 2009) in *Persea Americana*. This significant difference in rooting observed between the various types of nodes is due to internal plant hormones such as auxins and other cofactors which are concentrated in plant apex containing numerous meristematic cells (Hartmann et al., 2002). On the other hand, the results reported here are not in line with the one obtained *in vitro* multiplication by Ibañez et al. (2005) in *Vitis* (table grapevine cv. Napoleon), who found that the basal nodes produced better elongated shoots. Bona et al. (2005) noted no significant difference between apical, middle and basal nodes rooting in *Baccharis trimera.* Shoots derived from apical node showed a better growth with longer internodes. This best growth could be explained by easier nutrients uptake due to the presence of numerous roots initiated by these shoots. In contrary to this result, Shirdel et al. (2013) showed that shoots induced by basal node were longer than those produced by apical or middle nodes in *Rosa canina*. However, apical nodes produced low number of shoots than the others nodes. Similar result was reported by Mudoi et al. (2014) in *Bambusa nutans* Wall.ex. Munro. This low production of shoots by apical nodes could be explained by apical dominance. Indeed the high concentration of hormones such as auxins and other cofactors in apexes favors the growth of these apexes but inhibits lateral buds which enter in dormancy. Decapitation of apical node, stop this apical dominance and favors the lateral bud break and their growth on middle or basal node, this leads to the obtaining of more shoots on middle or basal node. Yadav and Singh (2012) and Aruna et al. (2012) also showed that apical nodes produced low number of shoots than the middle nodes respectively in *Glycyrrhiza glabra* L. and *Caralluma lasiantha.* On the other hand, Sá et al. (2012) noted no significant difference among the types of nodes in shoot number during micropropagation of *Hancornia speciosa*. In this study, no significant difference was observed in the multiplication rate whatever the nodes used. In contrary to this result, Nicoloso and Erig (2002) observed a great multiplication rate with the basal nodes in *Pfaffia glomerata.*

Conclusion

The study carried out here, revealed that apical nodes are the explants of choice for a rapid *in vitro* multiplication of *N. diderrichii*. These explants, not only initiated roots more rapidly but also expressing a fast growth of shoots with long internodes. The middle and basal nodes gave similar results

but their responses are less important than that of apical node concerning in vitroplants' growth and rooting.

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