Research Article

In Vitro Micropropagation: Effect of Leafy Nodes on Rooting and Growth of Plantlets of *Nauclea Diderrichii* (De Wild & T. Durand) Merrill

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ABSTRACT

The effect of leafy nodes of *Nauclea diderrichii* on the rooting and the growth of seedlings was studied *in vitro*. Three types of leafy nodes (leafless nodes, nodes with single leaf and nodes with two leaves) stemming from two months old of *Nauclea diderrichii*'s *in vitro* plants were tested using Woody Plant Medium (WPM). The number of roots and shoots was noted and the shoots and roots length of each seedling was measured after six weeks of culture. Nodes with two leaves grown on WPM without any phytohormone, gave seedlings with longer and more roots ($6.40^{c} \pm 1.57$ roots / plant) than those obtained with leafless nodes on the same medium ($4.35^{a} \pm 1.46$ roots / plant) or with leafless nodes even on a medium containing IBA at 0.1 mg/L ($5.60^{bc} \pm 1.35$ roots / plant). It was noticed that the plants length was also function of leaves presence on the nodes explants at the beginning of culture. So the positive influence of leaves presence at the beginning of culture on uninodal nodes of *N. diderrichii*, not only on the initiation and the growth of roots, but also on the growth of *in vitro* plants can reduce the cost of *in vitro* micropropagation of *N. diderrichii*, because it would not be necessary to use a very expensive exogenous auxins to improve plants rooting. Thus this study suggests that *N. diderrichii*'s leaves have enough endogenous auxins and others cofactors which can assure the *in vitro* plants' root initiation and growth.

Keywords: leafy nodes, rooting, growth, *N. diderrichii*, *in vitro* micropropagation.

INTRODUCTION

Leaves play an important role for vegetal species rooting.[1] The diverse nutrients (carbohydrates) synthesis takes place in leaves on a plant. Their contents allow a good growth of both the mother plant and the cuttings during the vegetative multiplication.[2] During the vegetative multiplication of plants, all the metabolic process necessary for the development of cuttings takes place in retained leaves on these cuttings.[3] In addition to this, their presence has also a considerable influence on rooting of cuttings, because of their ability to produce endogenous auxins, carbohydrates and their influence on water status of the cuttings.[4] According to[5], the presence of leaves on cuttings plays a significant role on roots initiation of many plant species. For[6], even though the presence of leaves plays an important role in rooting, it is necessary to consider certain factors such as leaf area and leaf number on cuttings. The presence of large leaves on cuttings has increased water loss and consequence the photosynthesis activity was reduced.[6] Therefore, it is necessary to pay attention in reducing of leaves area in order to not have effect on photosynthesis and the rooting of cuttings. The leafy or leafless cuttings influence the development of plants to regenerate. So during the present study the leafy and leafless cuttings of *Nauclea diderrichii* was tested *in vitro*. *N. diderrichii* is a forest plant species member of Rubiaceae family. Its regeneration *in situ* is laborious because of the dormancy of its seeds.[7] Despite the abundance of fruits produced by trees every year, the regeneration by seed is very low. Under the mother-trees, no young plant had been identified on the field.[8] This *in situ* deficit of seedling is very worrisome when we know that the species is overexploited for its very resistant wood against the attacks of fungus (*Coriolus versicolor*), Lyctus, termites (*Reticulitermes santonensis*) and marine borers by local sawyers.[9,10] Furthermore, some of its organs (barks) are used in traditional medicines.[11] Its *in vitro* regeneration can be a good alternative to supply a stock of seedling of plantation.[12]

The study carried out here, is consisted to determine the effect of leafy or leafless cuttings on *in vitro* rooting and the shoots development of seedlings, in order to identify the more suitable type cutting for rapid and massive production by *in vitro* vegetative micropropagation of *N. diderrichii*'s seedlings.

MATERIALS AND METHODS

Vegetal material used was constituted by uninodal leafless and leafy fragment of *N. diderrichii's in vitro* plants of two months old *in vitro* plants. Culture is initiated on Woody plant medium (WPM) without phytohormone or containing Indol-3-butyric acid (IBA) at 0.1 mg/L, both composed by[13] macroelements, 100 mg/L of myo-inositol, microelements and vitamins of[14]. This basic medium is supplemented by sucrose at 30 g/L rate 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 at the pressure of 1 bar during 20 minutes. Two months old plants are cut in uninodal leafless and leafy fragments by

constituting three groups of nodes: leafless nodes, nodes with single leaf and nodes with two leaves. On WPM without phytohormone the three types of nodes above are then put in culture and on WPM added with IBA at 0.1 mg/L, only leafless nodes are put in culture (photo 1). Tubes containing the different nodes are stored in a culture room with a photoperiod of 16 hours, at 27 ± 2°C and a light intensity of 120 $\mu Em^{-2}s^{-1}$. The light is supplied by fluorescent lamps.

Observations were performed during six weeks. At the end of every week, the number of roots, shoots, nodes was noted and the shoots and roots length were measured. Every measure was made on a population of twenty individuals and has been repeated twice. The results presented in this work correspond to those obtained after six weeks of culture. 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.

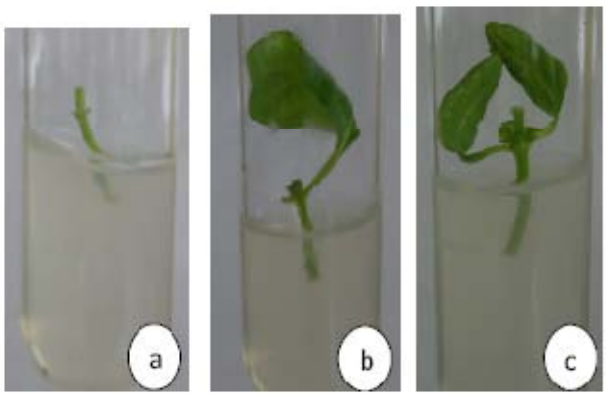

Photo 1: Different types of nodes used for culture, leafless node (a), node with single leaf (b), node with two leaves

RESULTS

The results obtained after six weeks of culture showed that the nodes with two leaves, developed better than allother types of nodes in culture. These nodes with two leaves, gave seedlings with longer internodes (photo 2). Their roots were longer and bigger with many secondary roots on primary roots.

The number of root (6.40^{c} ± 1.57 roots / plant) initiated by the nodes with two leaves was significantly different from that obtained with leafless nodes (4.35^{a} ± 1.46 roots / plant). Even in the presence of IBA at 0.1 mg/L, the number of roots obtained with the leafless nodes (5.60^{bc} ± 1.35 roots / plant) was significantly different from that obtained with leafy nodes cultured on medium without phytohormone. It was noticed that without exogenous auxins (IBA), the number of roots and their length by plant was a function of the presence of leaves on the nodal explants (Table 1).

Photo 2: seedlings stemming from development of leafless node (a), node with single leaf (b), node with two leaves (c)

⟷ = Length of internode

Table 1: Influence of leafy nodes on rooting of *N. diderrichii*'s plantlets

Types of nodes	Concentration of IBA in culture medium (mg/L)	Roots number/plant	Roots length/plant
Leafless nodes	0.00	4.35^{a} ± 1.46	2.55^{b} ± 0.56
Leafless nodes	0.10	5.60^{bc} ± 1.35	3.70^{a} ± 0.71
Nodes with single leaf	0.00	5.15^{ab} ± 1.57	3.70^{a} ± 0.71
Nodes with two leaves	0.00	6.40^{c} ± 1.57	4.38^{c} ± 1.05

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 $P < 0.05$.

Table 2: Influence of leafy nodes on shoots development of *N. diderrichii*'s plantlets

Types of nodes	Concentration of IBA in culture medium (mg/L)	Nodes number/plant	Shoots number/plant	Shoot length (cm)
Leafless nodes	0.00	$2.55^{b} \pm 1.69$	$1.10^{b} \pm 0.31$	$3.02^{b} \pm 1.46$
Leafless nodes	0.10	$3.90^{a} \pm 1.12$	$2.25^{a} \pm 0.91$	$5.26^{a} \pm 1.29$
Nodes with single leaf	0.00	$4.60^{a} \pm 1.90$	$2.40^{a} \pm 1.14$	$4.39^{a} \pm 1.86$
Nodes with two leaves	0.00	$4.30^{a} \pm 1.42$	$2.10^{a} \pm 0.91$	$7.65^{c} \pm 2.98$

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 plants growth and the multiplication rate were also a function of the presence of leaves on the nodes explants (Table 2). It was observed the calluses on the bottom of seedlings stemming from leafless nodes on medium containing 0.1 mg/L of IBA; but no callus was observed on seedlings stemming from the same type of nodes in culture on medium without IBA. Furthermore no callus was observed on seedlings stemming from the leafy nodes.

DISCUSSION

The results obtained during the present work, showed that the rooting and the shoots development of seedlings depended on leaves presence on the nodal explants. Indeed the roots number of seedlings stemming from nodes with two leaves at the beginning of culture was significantly different from that of seedlings obtained with the leafless nodes or the nodes with single leaf. This result is similar to that obtained *ex-situ* by[15-17], who showed that the presence of leaves on cuttings was necessary to obtain an optimal rooting of these cuttings respectively for *Khaya senegalensis*, *Momoridica charantia* and *Garcinia lucida*. The rooting of cuttings takes account not only of the presence of leaves, but also the leaf area. Indeed, during the present study, it was found that the nodes explants with two leaves gave vigorous roots and which number was significantly different from that obtained with nodes explants with single leaf. This result is in agreement with those of[18,4] respectively for *Shorea leprosula* and *Milicia excelsa* where they showed that the percentage of rooting of cuttings was affected according to the variation of the leaf area. On the contrary,[19,6] showed that the leaf area variation on cuttings respectively for *N. diderrichii* and *Terminalia spinosa* had little effect on the rooting percentage. On the other hand,[20] found that the retention of great leaf area on cuttings of *Lavandula dentata* was better for rooting than small leaf area. The reduction of the leaf area allows the reduction of the water loss by transpiration; however the retention of great leaf area on cuttings can allow the production of vigorous roots in a short time because the initiation and growth of root require a good oxygenation. Leaves serve as source of auxins and other necessary co-factors for the initiation and the improvement of rooting.[20] For other species on the other hand, it is the small leaf area which favors better rooting. It is the case for *Vitex doniana,*[1] *Lovoa trichilioides,*[21] *Prunus africanus*.[22] Lack of adequate leaf retention may be one of the reasons why some species are labeled as difficult to root.[20] The importance of leaf retention on cuttings has been observed also for *Pogostemon cablin*[23] and *Passiflora alata*[24] where these authors showed that the leafy cuttings gave a greater rooting percentage than leafless cuttings. For *Triplochiton scleroxylon* none leafless cutting was unable to root.[25] On the contrary, some species present an opposite response to leaf presence. For instance, *Ligustrum sinenses* leafless cuttings rooted better than leafy ones.[26] The best rooting obtained in the present work thanks to the presence of leaves, favored more the growth of seedlings. The similar result was found *ex-situ* by[27] for *Picralima Nitida*. This study has allowed to know that the presence of leaves on uninodal nodes of *N. diderrichii in vitro* micropropagation, has a positive influence not only on the initiation and the growth of roots, but also on the growth of *in vitro* plants' shoots. This best rhizogenesis may allow a good wean of plants when we know that the success of this weaning phase requires that plants have enough roots. The use of these leafy nodes explants can reduce the cost of *N. diderrichii*'s *in vitro* micropropagation because it would not be necessary to use exogenous growth hormones as auxins (IBA) recognized for their capacity of improvement of rooting of plants but which are very expensive. So this study suggests that the *N. diderrichii*'s leaves contain enough endogenous auxins and other cofactors which can assure the *in vitro* plants' roots initiation and growth.

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