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Full Length Research Paper

In vitro regeneration of *Neocarya macrophylla* (Sabine) Prance, wild fruit of Niger

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Neocarya macrophylla **is a spontaneous fruit species that constitutes the dominant population of Dallol Bosso in Southwestern Niger. In order to test the *in vitro* regeneration ability of this species, micro-propagation was applied to micro-cuttings from young almond seedlings. The Murashige and Skoog basal medium was enriched with several concentrations of naphthaleneacetic acid (NAA; 0, 0.5 and 1 mgL^{-1}) and 6-benzylamino purine (BAP; 0, 0.5, 1, 1.5, 2, 2.5 and 3 mgL^{-1}). The first results show that it is possible to obtain vitro-plants by vegetative propagation *in vitro* even at low doses. Thus, in this test, the overall recovery rate was 67.30%, the lag time was 11 days, and the recovery time was spread over 4 days. The statistical analysis at 5% significance threshold showed that there is no significant difference between the different treatments during the emergence of the buds and the neoformation of the leaves, but at the level of the elongation of the stems, the addition of these growth regulators has significantly impacted the results.**

Key words: *In vitro* regeneration, *Neocarya macrophylla*, micro-cuttings, growth regulators, Niger.

INTRODUCTION

Neocarya macrophylla (Sabine) Prance is a wild fruit tree of the Chrysobalanaceae family (Arbonnier, 2009). This woody tree, formerly known as *Parinari macrophylla* of the Rosaceae family, extends in Africa from the Guineo-Congolese region to the Sudano-Zambezian region, especially in the coastal savannahs of Senegal, Liberia, in the woodland of Southern Mali, Southern Niger and North Nigeria. The species grows on Sudanian and Guinean fringes, on forest and lowland areas and on sandy soils (Berhaut, 1967).

In Niger, its range extends the length of the Dallol Bosso to Fandou Beri and Bonkoukou, on the Dallol Maouri and Foga rivers, on the grounds of Goulbin Maradi, in the Goulbin Kaba to Kanan Bakaché and in the Korama (Dungas, Matameye, Magaria, and East Doungou) (Saadou, 1990).

More abundant in the Dallol Bosso Valley, this species keeps its green leaves especially all year (Sidikou, 1996, 1999). It is used in cooking, traditional pharmacopoeia, pagan religious practices, provides a useful fire wood and offers many services to the populations through the persistent shading it provides.

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It is a tree that produces fruits locally known as Gamsa, consumed and whose trade brings a substantial income to the local population (Balla et al., 2008) and possesses oleaginous properties (Tijani et al., 2010).

The production rate of the fruit estimated at 390.96 kg/tree in 2009 in Niger (Dan Guimbo et al., 2012) remains below the demand.

Although the regeneration of the tree is very important, this species is under increasing pressure, what explains the noticeable decrease of its aging population. This species is threatened as extinction (Saadou, 1990).

Despite its socio-economic importance, very few studies have been conducted on this species, hence the need for assisted regeneration, in order to avoid its very near extinction and perhaps to develop it. This regeneration is only possible with *in vitro* culture. Indeed, today, with plant biotechnology, it is possible to obtain healthy, greenhouse-transferable plants for the industrial production of seedlings of higher health level and high genetic homogeneity.

It is with this in mind that a micropropagation test of this species is proposed to vary the propagation modes and to plan a reintroduction of the same species so as to green the Dallol Bosso with *N. macrophylla* and increase fruit production and local income by producing thousands of seedlings.

The objective of this study, which is an introduction to the *in vitro* culture of *N. macrophylla*, is essentially aimed at testing the ability of the cuttings from young seedlings of this species to an assisted regeneration using micropropagation method.

MATERIALS AND METHODS

Source of transplants

The transplants used in these trials were cuttings of young *N. macrophylla* almond seedlings at seven weeks old, measuring of about 15 cm long and ±5 leaves (nodes). On each seedling, two thirds of the upper parts were used. The almonds are from the 2016 harvest provided by the National Forest Seed Center (CNSF).

Preparation of the culture medium

Distilled water (100 ml) was poured into a vial with Murashige and Skoog (1962) medium. The powder of the stock solutions was successively added in addition to sucrose (30 g) and the growth regulators to supplement the volume of distilled water at 1litre and to adjust the pH at 5.7 with HCl or NaOH (0.1 N). Agar (7 g with stirring and heating) was added and the solution dispensed into test tubes (25 ml/tube) and was sterilized.

Aseptic technique

All equipment was disinfected before use:

(1) The culture medium was sterilized in the autoclave at 121°C for 20 min at a pressure of one bar;
(2) The technical material (tongs, scalpels, etc.) was sterilised in an oven at 200°C for minimum of 2 h;
(3) The plant material was sterilised by taking the non-sterile plant material, washing it with water, then with soap and then rinsing it with distilled water. The micro-clippings were then transferred under a hood to be soaked in alcohol (70°) for 30 s and then in bleach (8°) for 10 min and then underwent three rinses with sterile distilled water for 15, 10 and 5 min, respectively.

Implementation

Under the horizontal laminar flow hood and in the vicinity of a ball sterilizer, the disinfected micro-clippings were cut into several fragments on blotting paper and planted in the culture medium using a sterile forceps. About 1.5 cm of explant carrying a single node endowed with a bud was placed in each tube and subsequently closed hermetically and was labelled. The tube was then placed in a culture chamber.

The emergence of the buds and the development of young seedlings took place in the culture chamber set at a temperature of 25 ± 2°C, with a photoperiod of 13/11-h under a light intensity of 3000 Lux.

Experimental apparatus

The experimental device consists of various combinations of concentrations of naphthaleneacetic acid (NAA; 0, 0.5 and 1 mgL^{-1}) and 6-benzylamino purine (BAP; 0, 0.5, 1, 1.5, 2, 2.5 and 3 mgL^{-1}) distributed randomly and conducted in four repetitions. Each repetition was represented by a cutting that carried a bud. For each medium combination, 4 transplants were transplanted.

The MS medium served as a control and, from it, two basal media were defined; in addition to the MS control T0, each medium consisted of six treatments (Table 1):

MS1 = MS supplemented with 0.5 mgL^{-1} of NAA and 0.5, 1, 1.5, 2, 2.5 and 3 mgL^{-1} of BAP.
MS2 = MS supplemented with 1 mgL^{-1} of NAA and 0.5, 1, 1.5, 2, 2.5 and 3 mgL^{-1} of BAP.

Parameters

The selected parameters evaluated are:

(1) The emergence of the bud which was characterized by: (i) the recovery time or latency time, that is, the time that elapses between the sowing of the micro-clipping and the appearance of the first bud; (ii) the duration of recovery or time that elapsed between the appearance of the first bud and that of the last bud; (iii) the recovery rate, which is the ratio between the number of micro-clippings giving buds and the total number of micro-cuttings transplanted;
(2) The elongation of the stem;
(3) The number of leaves per stem.

Statistical analysis

In order to highlight the effect of hormones on bud recovery and identify the best combination for elongation of stems and leaf formation, statistical analysis of these results was performed using the XL Stat 7.1 software. The test of Newman and Keuls, at the threshold of 5% significance was used to classify the averages.

Table 1. Codification of the different treatments.

Medium	Code	NAA (mgL^{-1})	BAP (mgL^{-1})
MS	T0	0	0
MS1	T1	0.5	0.5
	T2		1
	T3		1.5
	T4		2
	T5		2.5
	T6		3
MS2	T1'	1	0.5
	T2'		1
	T3'		1.5
	T4'		2
	T5'		2.5
	T6'		3

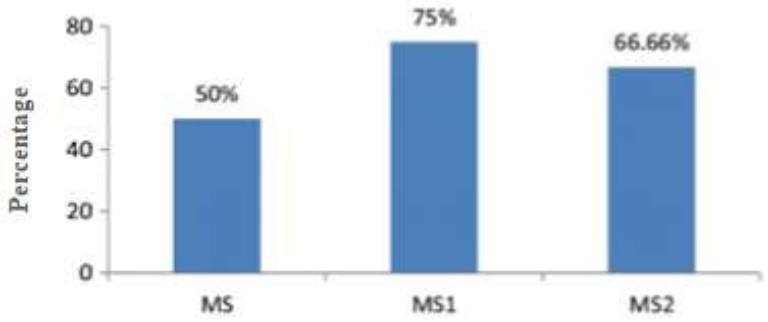


Figure 1. Percentage of the emergence of the bud by culture medium.

RESULTS AND DISCUSSION

At the end of this experiment which was done six weeks at *N. macrophylla*, the following results were recorded.

The emergence of the bud

The recovery time for the emergence of the bud (Figure 1 and Photo 1) took place 11 days after the culture of micro-clippings for all the treatments. This recovery was spread over 4 days. This positive response can be explained by the age of the mother plants used which is young and therefore more reactive.

In a similar study with *Maerua crassifolia,* Diata (2008) noted a lag time of 12 days. Indeed, Gitonga et al. (2008) have shown that the age of transplants has an influence on their performance.

The addition of growth hormones in the culture medium has greatly favoured the emergence of the bud.



Photo 1. Budding occurred after 11 days.

The overall recovery rate for micro-clipping is 67.30%. The percentage of success of this regeneration varied

Table 2. Newman-Keuls (SNK)/Analysis of differences between groups with a 95.00% confidence interval.

Medium	Difference	Reduced difference	Critical value	Pr.> Diff	Significant
MS1 ~ MS	0.210	1.176	2.668	0.489	No
MS1 ~ MS2	0.118	0.734	-	-	No
MS2 ~ MS	0.092	0.533	-	-	No

Table 3. Classification and grouping of non-significantly different groups.

Medium	Average	Group
MS1	0.435	A
MS2	0.317	A
MS0	0.225	A

according to the combinations of phytohormones used. Control MS gave 50% response. MS1 gave a more strong response (75%) while MS2 gave a 66.66% response.

These results thus inform us on one hand the quality of the plant material and on the other hand the action of the doses of hormones on the growth of the vitro-plants.

Houédjissin et al. (2015) pointed out that the difference of the recovery and development of the apex of *Pentadesma butyracea* observed between the culture medium used (B5, MS and WPM) would certainly result from the difference in their mineral composition especially the K^+, NO^{3-} and NH^{4+} ions.

Also, Zuraida et al. (2015) obtained a rate of 80% over six weeks by adding to the MS medium 3 mgL^{-1} of BAP with micro-clippings of *Plectranthus amboinicus*.

Ndoye et al. (2003) by studying micro-cuttings of *Balanites aegyptiaca* with a combination of 1 mgL^{-1} of BAP and 0.1 mgL^{-1} NAA after 4 weeks recorded 100% recovery.

These results show that one species-friendly medium may not be suitable for another species.

The average elongation of stems

Mean elongation of stems according to auxin dose

Statistical results on the effect of dose of auxin on the elongation of stems are shown in Tables 2 and 3.

These two tables show that the responses obtained are not significantly different; the probability (0.48) being greater than 0.05. The applied dose of auxin therefore does not significantly affect the emergence of the bud. All three media are classified in the same group A with the leading MS1 medium that has an average of 0.435.

Mean elongation of stems according to the combination of auxin and cytokinin

The resumption of vegetative activity characterized by the elongation of the stem is as shown in Figures 2 and 3.

This height improved significantly in the MS1 medium and the seedlings reached an average size of 0.9 cm in the same time period. The emergence of the stem axis was not observed in all germinated cuttings, in fact, the micro-cuttings of the T5 treatment experienced necrosis just after their emergence.

The behaviour of the transplants was favourable in the presence of the growth regulators as in their absence. However, in the control (T0), the average height of the seedlings did not exceed 0.2 cm after 42 days of growth culture.

From the 37th day, all micro seedlings ceased growing; those of the T4 treatment even experienced a regression. Experimentally, the best result was observed with the T3 treatment where some stems reached up to 2 cm in length. The analysis of curves on MS2 shows that the addition of growth regulators has also had a positive effect on the elongation of the stems. This is relatively higher with the combination T2' which gave an average size of 0.8 cm.

As in the MS1 medium, the MS2 from the 37th day revealed that all growth in the length of the stem remained stationary for all the treatments except for T1' which experienced a slight increase. T3 seedlings suffered necrosis just after emergence of the bud. The maximum length (Photo 2) obtained during this test is 1.8 cm with the combination T4'.

The Newman and Keuls test of the influence of hormonal combinations on the elongation of the stem classifies the treatments into four groups (A, B, BC and C).

Although the elongation of stems was very low in all the tests; the addition of growth regulators had a positive influence, thus there are significant differences in stem growth according to the combination applied and the most significant is the T3 combination. The averages between 0.717 and 0.608 belonging to treatments T3, T4 ', T4, T2 and T2' are classified in group A with T3 in the lead. Treatments T6', T1 and T6 are classified in group

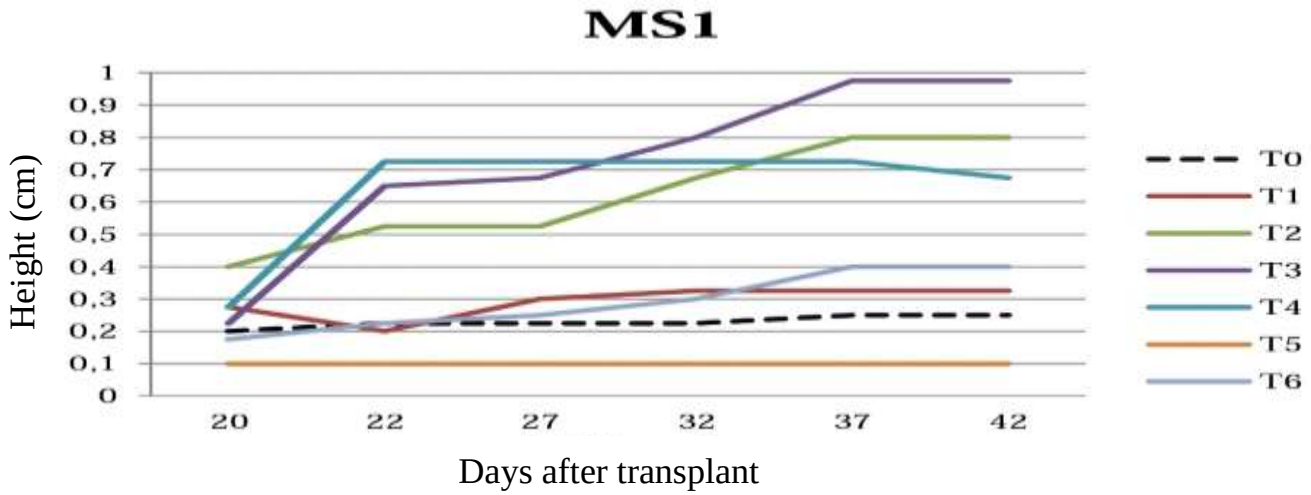


Figure 2. Average elongation of stems on MS1 medium (Effect of doses (0 and 0.5 mg.L^{-1}) of NAA and (0, 0.5, 1, 1.5, 2, 2.5, and 3 mg.L^{-1}) of BAP).

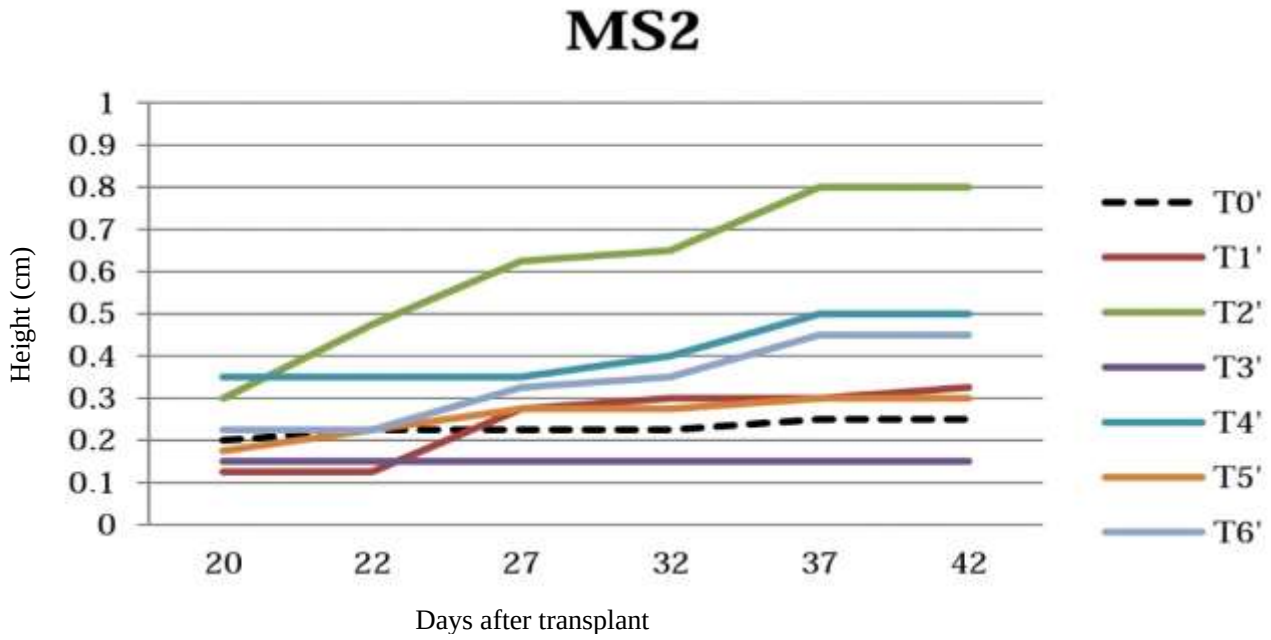


Figure 3. Mean elongation of stems on MS2 medium (Effect doses (0 and 1 mgL^{-1}) of ANNA and (0, 0.5, 1, 1.5, 2, 2.5 and 3 mgL^{-1}) of BAP).

B with averages of 0.338 to 0.292. The B and C groups with averages ranging from 0.263 to 0.150 includes the treatments T5 ', T1', T0 and T3', respectively. The last T5 treatment is placed in the group C.

However, Ndoye et al. (2003) with *B. aegyptiaca* after 4 weeks was found to be 2.29 cm in length with a combination of 2.5 mg.L^{-1} of BAP and 0.1 mg.L^{-1} of NAA in MS medium. For Rancillac and Lafargue (1981), benzyladenine was more effective than kinetin in inducing caulogenesis. A concentration between 2 and 4 mg.L^{-1} is the most favourable at the sea level. Beyond 30 days, growth in length of shoots stopped for the majority of treatments (except for T4 and T1') which justifies the need to change the medium after one month in culture as nutrients are exhausted.

Le Bellec (2017) attests that the first transplanting which takes place systematically 4 weeks after cultivation makes it possible to renew the culture medium which



Vitro-plant, 16 days old Vitro-plant, 21 days old

Photo 2. Height of the vitro-plants of *Neocarya macrophylla*.

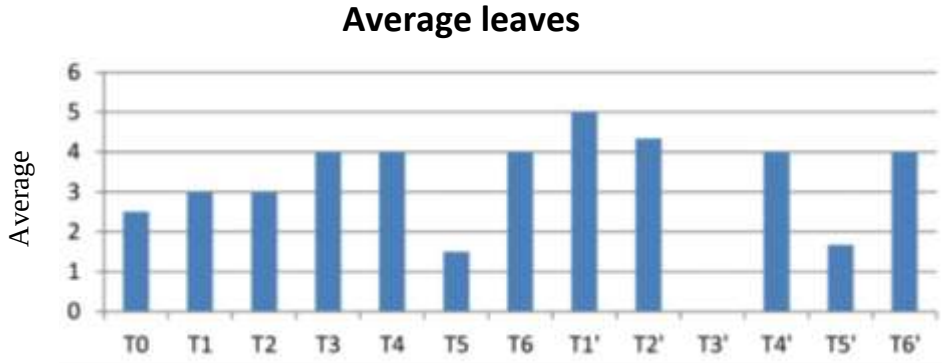


Figure 4. Medium shade of new leaves formed by treatment.

begins to be exhausted and which furthermore sometimes contain inhibiting substances released by the transplant itself during the growth of the bud.

The necrosis observed at T5 level are mainly due to lesions caused by sterilizing agents and transfer stress. The low percentage of T3' could also be the result of this stress.

The middle shade of new leaves formed by stem

After six weeks of culture, the results (Figure 4) show that all combinations are favourable to leaf formation except for one; therefore, the addition of cytokinin (BAP) in the culture medium significantly improved leaf formation by the vitro-plants.

Figure 4 shows that the responses to leaf formation differ according to the hormonal combinations.

The average number of leaves recorded in the control (T0) is 2.5. This number has constantly evolved from 3 to 5 leaves on average in MS1 and MS2 media except for T5, T3' and T5' treatments (where the minimum number of leaves has been noted). It was higher with T1' treatment (5 leaves on average).

Treatments T3, T4, T6, T2', T4' and T6' all gave almost the same number of leaves (4 on average). This constancy may be related to the purity of the species; micro-plants do not absorb more than their needs for nutrition.

Overall, the MS1 medium was more consistent and more favourable to leaf formation. From 0.5 to 1 mgL^{-1}, the seedlings give 3 leaves on average; but with up to 3 mgL^{-1} they give up to 4 leaves on average per micro-plant after 42 days.

These results are similar to those found by Adane (2013), with meristems of the Giant Cavendish plantain (*Musa* species) in a combination of 5 $mg.L^{-1}$ of BAP and 0.5 $mg.L^{-1}$ of NAA recording a greater mean leaf length of 4.33 after 60 days.

Gbadamosi and Sulaiman (2012) noted for their part up to 6 leaves on average at *Irvingia gabonensis* by combining 0.05 mgL^{-1} BAP, 0.05 mgL^{-1} kinetin (KIN), 0.05

Table 4. Analysis of variance.

Source variations	Sum squares	Degree of freedom	Average squares	F	Probability	Value critical for F
Between groups	38.91666667	12	3.24305556	0.756152	0.68335204	2.475312973
Within groups	64.33333333	15	4.28888889	-	-	-
Total	103.25	27	-	-	-	-



Photo 3. Development of bud 21-day old leafy plants.

mgL^{-1} AIB and 10.0% coconut water in ¼ MS. Analysis of variance of the number of sheets is shown in Table 4.

The analysis of variance on the average number of leaves issued by the stems gave a probability of 0.68; this being greater than 0.05, the effect of cytokinin on leaf formation is not significant in this case. Photo 3 shows the T3 treatment leaves.

Overall, the T3 combination appears the best of all and the most economical *in vitro* for regeneration of micro-clippings of this species; a concentration of 0.5 mgL^{-1} NAA and 1.5 mgL^{-1} BAP is sufficient to induce the formation of stems with leaves at the buds in *N. macrophylla*. In this trial, callus formation or root formation was not observed.

Conclusion

This study shows that the regeneration of *N. macrophylla* is surely possible using the in vitro culture technique, because it has successfully produced vitro-plants from micro-clippings taken from young seedlings. In this work, challenge was encountered when all the technical advantages of *in vitro* culture for the successful production of plants, which could be used for its propagation and domestication are put at the service of this species. Thus, efforts have been focused on the culture medium to propose the best combinations in terms of hormones.

The basal medium is Murashige and Skoog, the hormones used are NAA (auxin) and BAP (cytokinin). These tests led to the conclusion that:

(1) Regeneration *in vitro* of *N. macrophylla* can be done with young cuttings;
(2) The addition of hormones has significantly improved bud recovery capacity (up to 75% for MS1);
(3) Stem elongation and leaf formation increased considerably with the addition of cytokinin in the medium.

For the remaining of this preliminary work and the success of micro-propagation *in vitro* of *N. macrophylla*, it is necessary to extend research to other combinations, which would favour rhizogenesis, because this phase will depend on the acclimatization and consequently the

success of the propagation of this plant which is heavily exploited in the Dallol Bosso region.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Adane GD (2013). Effects of different combinations of BAP (6-benzyl amino purine) and NAA (naphthalene acetic acid) on multiple shoot proliferation of plantain (Musa spp.) cv. Matoke from meristem derived explant. Academia Journal of Biotechnology 1(5):071-080.

Arbonnier M (2009). Arbres, arbustes et lianes des zones sèches d'Afrique de l'Ouest.3è édition revue et corrigée. Versailles, MNHN QUAE ÉDITIONS 573 p.

Balla A, Barage M, Larwanou M, Adam T (2008). Le savoir-faire endogène dans la valorisation alimentaire des fruits du pommier de Cayor (*Neocarya macrophylla*) au Niger. Bulletin de la Recherche Agronomique du Bénin 59:1-7.

Berhaut J (1967). Flore du Sénégal, 2ème édition plus complète avec les forêts humides de la Casamance 485 p.

Dan Guimbo I, Larwanou M, Mahamane A, Ambouta KJ-M (2012). Production fruitière de *Neocarya macrophylla* (Sabine) Prance, espèce ligneuse alimentaire du Niger. Journal of Applied Biosciences 60:4388-4393.

Diata S (2008). Modes de propagation d'un ligneux fourrageux sahélien, *Maerua crassifolia* Forsk. Thèse de doctorat en biologie végétale. Faculté des sciences et techniques. Université Cheikh Anta Diop de Dakar 103 p.

Gbadamosi IT, Sulaiman MO (2012). The Influence of Growth Hormones and Cocos nucifera Water on the In Vitro Propagation of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill. Nature and Science 10(9):53-58.

Gitonga LN, Kahangi EM, Gichuki ST, Ngamau K, Muigai AWT, Njeru ES, Njogu N, Wepukhulu S (2008). Factors influencing the in vitro shoot regeneration in Macadamia integrifolia. African Journal of Biotechnology 7(22):4202-4207.

Houédjissin SS, Dangou SJ, Azokpota P, Cacaï G, Agbidinoukoun A, Hounhouigan DJ, Ahanhanzo C (2015). Régénération in vitro de l'arbre à Suif (Pentadesma Butyracea Sabine), une espèce ligneuse à usages multiples (Lum) vulnérable au Benin. European Scientific Journal 11(21).

Le Bellec F (2017). La culture du vanillier Cirad. La recherche agronomique pour le développement 30 p.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bio assays with Tobacco tissue culture. Plant Physiology 15(3):473-497.

Ndoye M, Diallo I, Gassama/Dia YK (2003). In vitro multiplication of the semi-arid forest tree, *Balanites aegyptiaca* (L.) Del. African Journal of Biotechnology 2(11):421-424.

Rancillac M, Lafargue P (1981). Perspectives d'application des cultures d'organes in vitro à la multiplication végétative du Pin maritime, *Pinus pinaster* Sol. Annales des Sciences Forestières, INRA/EDP Sciences 38(1):55-70.

Saadou M (1990). La végétation des milieux drainés nigériens à l'est du fleuve Niger. Thèse de doctorat es sciences naturelles, Faculté des sciences, Université Abdou Moumouni de Niamey 395 p.

Sidikou RDS (1996). Projets d'Amélioration et de Valorisation de deux fruitiers (un cultivé- un spontané) au Niger. Journées biologiques - Communication présentée aux journées biologiques de l'institut National de Recherche Agronomique du Niger (INRAN) du 25 au 26 mars 1996. Niamey, Niger, INRAN.

Sidikou RDS (1999). Contribution des biotechnologies végétales à la sécurité alimentaire en zones sahéliennes. Dans « Biotechnologies, Amélioration des Plantes et Sécurité Alimentaire », (Actes des 6èmes journées scientifiques du Réseau AUPEL UREF « Biotechnologies Végétales: Génie génétique des Plantes », Université de Paris Sud, Orsay, Juillet 1997:113-122.

Tijani A, Issoufou A, Mohamed TK, Kexue Z, Huiming Z (2010). Chemical and Nutrient Analysis of Gingerbread Plum (*Neocarya macrophylla*) Seeds. Advance Journal of Food Science and Technology 2(4):191-195.

Zuraida AR, Erny S, Mohd N, Mohd SMA, Razali M, Ayu NO (2015). In Vitro Micropropagation of a Valuable Medicinal Plant, *Plectranthus amboinicus*. American Journal of Plant Sciences 6:1091-1097.