First results about micropropagation of *Anacardium occidentale* by tissue culture.

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FIRST RESULTS ABOUT MICROPROPAGATION OF *ANACARDIUM OCCIDENTALE* BY TISSUE CULTURE
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ABSTRACT - The true to type propagation of *Anacardium occidentale in vitro* starting from nodes has been studied. Establishing culture has been difficult for certain clones due to the presence of phenolic compounds. The nodes were induced on a Lepoivre medium containing 2iP and GA_3. Proliferation was stimulated by the two phase technique which includes adding BAP to the growth medium for three days. Elongation is obtained in a shaked liquid medium. IBA-shoot pretreatment followed by a 10-days period of darkness allows rooting. Acclimatation has not caused problems.

PREMIERS RESULTATS SUR LA MICROPROPAGATION DE L'*ANACARDIUM OCCIDENTALE* L. PAR CULTURE DE TISSUS
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RESUME - La multiplication conforme d'*Anacardium occidentale in vitro* à partir de noeuds a été étudiée.
L'établissement des cultures a été difficile pour certains clones dû à la diffusion de composés phénoliques.
Les noeuds ont été induits sur un milieu Lepoivre contenant de la 2iP et la GA_3. La prolifération a été stimulée par la technique deux phases comprenant l'adjonction de la BAP au milieu de culture pendant 3 jours. L'allongement est obtenu en milieu liquide agité. Un prétraitement de pousses à l'IBA et une période d'obscurité de 3 jours permet l'enracinement. L'acclimatation n'a pas posé de difficulté.

INTRODUCTION

Cashew tree is an important nutritive and reforestation ressource for several tropical countries, especially in Eastern and Western Africa, in Brazil and in India.

The existing cashew plantation is raised by seedling and is heterogenous. The use of clones is thus necessary to establish new commercial plantations.

Vegetative multiplication by cuttings (Nageswaro *et al.*, 1988), by airlayering (Nagabhushanam *et al.*, 1979) and by grafting (Nagabhushanam *et al.*, 1979 ; Swake *et al.*, 1986 ; Seshadri *et al.*, 1986 ; Venketaras, 1981) has been investigated. But no reliable technique, at the same time simple and economical, has been fully satisfactory.

In *in vitro* cultures, very recent studies concerning the regeneration of adventitious buds (Ninan *et al.*, 1989 ; Philips, 1984) have been carried out. Only the cotyledones have enabled to obtain plantlets (Philips, 1984). Trials of calli culture have also been carried out (Ninan *et al.*, 1983).

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In this paper, we reported the first results of true to type micropropagation of cashew trees from nodes.

The study started in October 1986, with the initiative of the Deutsche Gesselschaft für Technische Zusammenarbeit (GTZ).

MATERIAL AND METHODS

Establishing parent plants in greenhouses.

The nuts originating from Senegal and Côte d'Ivoire are sorted through, they are treated with a 36% acetone solution for two hours and are then placed on the surface of the soil mixture with the peduncle tie facing upwards.

The soil mixture contained 1/3 sand.

Establishing the *in vitro* culture.

Nodes segments were taken from the herbaceous part of seedlings 6 to 15 months-old.

After leaves removing, the shoots are soaked in a solution of benlate (4 g/l) for 10 minutes, they are then dipped in calcium hypochlorite 9% for 25 minutes and rinced in sterile water.

The basal initiation medium consist of Lepoivre macro-elements, Nitsch microelements and Jacquiot vitamins.

Influence of Kn, 2iP, BAP, Thiadiazuron, NAA and GA3 was studied by adding the hormones in various combinations :

M1 KN 1 mg/l	+ NAA 0,1 mg/l
M2 KN 2 mg/l	+ NAA 0,1 mg/l
M3 2iP 2 mg/l	+ NAA 0,1 mg/l
M4 2iP 4 mg/l	+ NAA 0,1 mg/l
M5 2iP 2 mg/l	+ NAA 1 mg/l
M6 2iP 2 mg/l	+ NAA 0,01 mg/l
M7 2iP 2 mg/l	+ GA3 0,5 mg/l
M8 BAP 2 mg/l	+ GA3 0,5 mg/l
M9 Thiadiazuron 0,5 mg/l	+ GA3 0,5 mg/l

10 plants were used for this trial and 9 nodes were taken per plant. The analysis of the results was based on the development of the axillary bud.

Trials during the proliferation stage.

The leafless shoots are placed in a basal medium (Table 1) containing 0 and 2 mg/l of BAP.

To stimulate proliferation, the explants are immerged during 0, 3 or 30 days by adding a solution of BAP (5 mg/l) following the 2 phases technique described by Viseur (1985).

In parallel, we compared the behaviour of shoots partly immerged with the induction medium (Table 1).

Elongation of the axillary buds.

The explants with the axillary buds are transferred into a shaked liquid elongation medium (Table 1).

Rooting and acclimatation.

Rooting is carried out on vigorous shoots of ± 3 cm. It includes soaking the shoots in a solution of IBA 4 mg/l for 16 hours and then growing them on in an agar-medium containing IBA (0, 1 and 2 mg/l).

A treatment in darkness for 3 and 10 days has been studied.

The plants were acclimated on Jiffy 7. A pulverisation with a solution of TMTD (Thirame) 1% prevents all fungal development.

RESULTS AND DISCUSSION

The germination rate of the seeds is of 95 %.

Our method of disinfection enables to obtain an average rate of 74% healthy explants.

TABLE 1 - Composition of used culture media (mg/l).

	Induction	Multiplication	Elongation	Rooting
Macroelements				
NH_4NO_3	400	1.200	400	825
KNO_3	1 800	1 900	1 800	950
$Ca(NO_3)_24H_2O$	1 200	710	1 200	
$MgSO_47H_2O$	360	370	360	185
KH_2PO_4	270	170	270	85
$NaNO_3$		450		220
$CaCl_22H_2O$				
Microelements	MS (1962)	MS	MS	MS
Vitamins	MS	MS	MS	MS
BAP	-	2	-	-
2iP	2	-	2	-
IBA	-	-	-	2
GA_3	0,5	-	0,5	-
NAA	-	0,001	-	-
Ascorbic acid	100	100	-	-
Glucose	30 000	30 000	30 000	30 000
Agar Merck	7 000	7 000	-	7 000
pH	5,5	5,5	5,5	5,5

ABREVIATIONS :
GA3 : gibberellic acid
BAP : 6-benzylaminopurine
IBA : 3-indolebutyric acid
KN : kinetin
2iP : N^6 (2-isopentyl) adenin
NAA : naphtalen acetic acid

For certain clones, the establishment of the culture is made difficult or almost impossible due to the importance of the diffusion of phenolics compounds and the subsequent necrosis of the explants (Table 2, plants 5 and 9). Ascorbic acid is added to the growing medium to limit the

TABLE 2 - Development of the axillary bud.

Media \ Plant	1	2	3	4	5	6	7	8	9	10	a	b	c
M1	b	a	a	a	i	b	a	a	c	b	5	3	1
M2	a	a	a	a	c	a	a	a	a	a	9	0	1
M3	a	a	a	b	i	b	a	b	c	a	4	3	1
M4	a	c	a	b	c	i	a	b	c	a	4	2	3
M5	c	c	c	c	c	c	c	c	c	c	0	0	10
M6	b	c	a	a	a	a	a	a	a	a	8	1	1
M7	a	a	a	b	a	a	a	a	b	a	8	2	0
M8	b	b	c	c	c	b	a	b	i	i	1	4	3
M9	c	c	c	c	c	c	c	c	c	c	0	0	10

Legend : a : bud with foliar development
b : open bud without foliar development
c : no axillary development or necrosis
i : culture with an infection

TABLE 3 - **Development of axillary shoots after 0, 3 and 30 days of BAP immersion (5 mg/l), in presence or in absence of BAP in the solid phase.**

Media \ BAP	5 mg/l		
	0	3 days	1 month
A : BM+ BAP 2 mg/l	3/6*	19/6	necrosis
B : BM without BAP	0/5	10/6	necrosis

* - numerator : number of explants
denominator : number of shoots.

TABLE 4 - The influence of the shaked liquid medium on the number of axillary buds which are elongated and vitrified (number of explants : 15).

Elongation \ Vitrification	vitrified	normal	total	elongation rate
+	5	10	15	15/77 : 19,5%
-	40	22	62	62/77 : 81,1 %
total	45	32	77	
Vitrification rate	45/77 : 59%	32/77 : 41%		

phenol oxydation.

The M2, M6 and M7 mediums enable to obtain the highest success rates. The presence of NAA at 1 mg/l of Thiadiazuron 0,5 mg/l induces a total necrosis of the nodes for all the plants. Kinetin and 2iP enable to obtain similar developments of axillary buds but in the presence of 2iP, the leaves obtained are more vigorous. GA_3 stimulates elongation of the axis whereas BAP enables the opening of the bud but limits the development of the axis.

A shoot of ± 2 cm is obtained after 2 months growing on an induction medium (M7) (Photo 1). It is then transferred on a multiplication medium.

The development of axillary buds in the proliferation medium is considerably improved by adding BAP in the liquid phase (Table 3). The presence of hormones in the solid medium also encourages axillary development (Table 3). As BAP is a cytokinine which is not very mobile in the explant and which encourages proliferation, it is normal that applying a liquid solution stimulates the formation of axillaries (Photo 2).

But the continuous presence of a solution of BAP (1 month) induces a toxicity which marks itself by a total necrosis of the shoots.

The foliar development of young leaves is strongly stimulated in a two phase medium where induction medium is used as liquid medium.

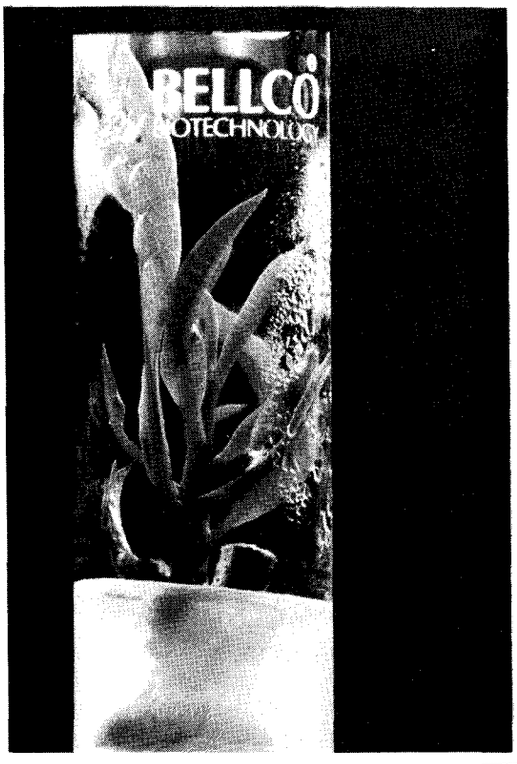

Photo 1 - Development of axillary bud on induction medium.

A shaked liquid medium enables the elongation of certain axillary buds, but also can induce some vitrification (see Table 4).

Other elongation trials involving the addition of GA3 have been carried out but none have enabled to improve the elongation rate.

The presence of IBA (2 mg/l) combined with a treatment in darkness for 10 days has enabled to obtain a rooting rate of about 30%. The roots are formed about ten days after coming out of darkness, they are not very numerous (2-3 per explant) and they diffuse a few phenol compounds.

The rooted plantlets have new leaves after about ten days.

On the contrary of the studies of Philip (1984) which concern adventitious regeneration from cotyledons, the method described here above is a technique of true to type multiplication from preformed buds.

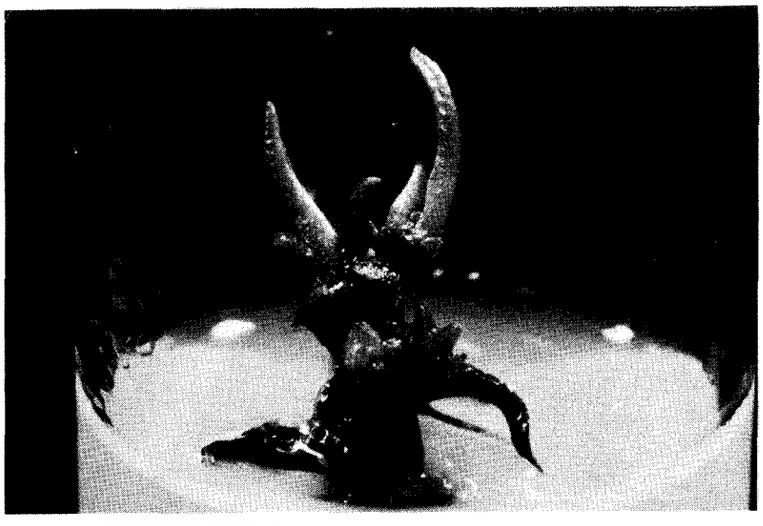

Photo 2 - Development of axillary buds in proliferation phase.

Photo 3 - Acclimated plant.

Although applied up to now only on juvenile material, our conditions of multiplication by axillary buds seem promissing for clonal propagation of cashew trees selected in orchards for their fruit production.

CONCLUSIONS

Our trials of *in vitro* cultures have led to a true to type plantlets from axillary branching and to obtaining plantlets in greenhouses. The study has been carried out on juvenile material.

The state of the initial material as well as the state of the *in vitro* shoots plays an important role in the success of each phase.

Two major elements have to be improved : the elongation rate and the rooting rate.

Nevertheless, we think that this technique is a starting point for further research on massal propagation of clonal cashew tree.

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ERSTE ERGEBNISSE DER MIKROSKOPISCHEN VERMEHRUNG VON *ANACARDIUM OCCIDENTALE* L. MITTELS GEWEBEKULTUR.

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KURZFASSUNG - Die konforme *in vitro*-Vermehrung von *Anacardium occidentale* anhand von Knoten wurde untersucht.
Bei manchen Klonen war die Einrichtung von Kulturen wegen der Streuung der Phenolkomponenten schwierig.
Die Knoten wurden auf einem Lepoivre-Nährmedium mit 2iP und GA_3 induziert. Stimuliert wurde die Vermehrung mit der Zweiphasentechnik , wobei dem Zuchtmedium drei Tage lang BAP zugesetzt worden ist. Die Verlängerung erhält man im bewegten flüssigen Medium. Die Wurzelbildung erfolgt nach Vorbehandlung der Triebe mit IBA und dreitägiger Verdunklung.

PRIMEROS RESULTADOS DE LA MICROPROPAGACION DEL *ANACARDIUM OCCIDENTALE* L. POR CULTIVO DE TEJIDOS.

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RESUMEN - Se ha estudiado la multiplicación conforme de *Anacardium occidentale in vitro* a partir de nudos.
El establecimiento de cultivos para ciertos clones ha sido difícil debido a la difusión de compuestos fenólicos.
Los nudos se han inducido sobre un medio Lepoivre que contiene 2iP y la GA_3. La proliferación se ha estimulado por la técnica dos fases que comprende la añadidura de la BAP en el medio de cultivo durante 3 días. El alargamiento se obtiene en medio líquido agitado. Un pretratamiento de brotes con el IBA y un período de oscuridad de 3 días permite el enraizamiento. La aclimatación no ha planteado dificultad.