EFFECT OF CYTOKININS ON MICROPROPAGATION OF TEAK (*TECTONA GRANDIS* L.) GROWN IN COTE D'IVOIRE

ABSTRACT

Teak (*Tectonagrandis* L.) is a timber tree highly prized on world markets for its stability and durability. This hardy species has good initial growth and is very important for the reforestation of Côte d'Ivoire. However, teak regeneration potential by seed or cutting still very weak. This situation is a major constraint to planting material production in quantity and quality. The aim of this study is to improve the production of teak seedlings for orchard renewal using *in vitro* culture methods. Mature teak seeds were surface disinfected and sown on Murashige and Skoog (MS) basal medium supplemented with different concentrations of Benzyl-aminopurine (BAP), Kinetin and Thidiazuron (TDZ). The shoot tip explants and nodal segments were taken from *in vitro* germination and placed on the same medium used for seeds germination. Leafy shoots obtained after four weeks of culture were excised and transferred individually on different strength of McCown Woody plant medium supplemented with various concentrations of AIB for rhizogenesis. Teak seeds germination started seven days after sowing. BAP and Kinetin exhibited highest percentage of normal seedlings (83.46% and 74.14% respectively). Higher seedlings (7.48 cm) were obtained on medium supplemented with 0.5 mg/L kinetin. The shortest bud break time (7.43 days) was observed on medium containing 0.5 mg/L BAP. Leafy shoot production was optimal (5 leafy shoots per explant) with the nodal segment on culture medium containing 2 mg/l BAP. Half strength of McCown Woody plant mediumcontaining 60 g/L sucrose and 4 mg/l AIB induced the highest rooting percentage (73%) and roots number per plant (03) natural environment.

Keywords: Tectonagrandis, in vitro germination, Cytokinins, leafy shoots, rooting

INTRODUCTION

Sustainable forest management is now a major concern, particularly in tropical regions which are facing alarming deforestation [1].In Côte d'Ivoire, forest cover has been declining since the beginning of the 21st century, falling from 5.9 million hectares in 2000 to 3.6 million hectares in 2015 [2].Current forest cover in Côte d'Ivoire is estimated at less than 2 million hectares [3]. The main factors responsible for the gradual disappearance of forest resources are shifting agriculture, which tends to increase in area as the population grows, firewood production, bush fires, timber marketing and livestock farming. To make up for this shortfall, SODEFOR has initiated the reforestation of hundreds of hectares of forest with various species, of which teak accounts for around 40 % [4]. Teak has enjoyed particular success due to its global market demand and, above all, because it is a suitable species for reforestation.

However, the low germination and cutting rates of teak do not encourage the production of planting material in sufficient quantity and quality to restore the forest cover [5]. To overcome these constraints in teak, the use of in vitro culture techniques appears to be an alternative solution. However, [6] obtained an in vitro germination percentage of 60 % on MS medium supplemented with BAP (2.2 µM).

An average of around 4 shoots per nodal segment was obtained a Murashige and Skoog (Murashige and Skoog, 1962 [9] medium reduced by half and supplemented with BAP (1.5 mg/L), AIB (0.01 mg/L) and GA3 (0.1 mg/L) [7]. Similarly, rhizogenesis was promoted by adding 0.25 mg/l AIB and 25 mg/l β-cyclodextrin to the MS medium, which resulted in the formation of 9.22 roots per vitroplant [8]. The objective of this work is to contribute to the restoration of Côte d'Ivoire's forest cover by developing an *in vitro* teak regeneration protocol.

MATERIAL AND METHODS

1. Plant material

The plant material consists of teak mature seeds collected by the forestry company (SODEFOR) in Adzopé in the south-east of Côte d'Ivoire.

2Methods

2.1 *In vitro* germination of Teak seeds

2.1.1 Preparation of *in vitro* germination medium

Murashige and Skoog (1962)[9]basal medium containing 30 g/L of sucrose was used for germination. This medium was supplemented with different concentrations of Benzyl-aminopurine (BAP) (0.5, 1, 2 and 4 mg/L), kinetin (0.5, 1, 2 and 4 mg/L) and Thidiazuron (TDZ) (0.01, 0.1, 0.25 and 0.5 mg/L), making up twelve culture media. The control medium was devoid of cytokinins. The medium pH was adjusted to 5.7 using NaOH (0.1 N) or HCL (0.1 N), then solidified with 8 g/L agar and sterilized at 121°C for 20 minutes at a 1 bar pressure.

2.1.2 Preparation, disinfection and teak seeds sowing

The fruit was removed from its shell by hand and carefully crushed to release the seeds. The seeds were then placed on water-soaked filter paper to prevent drying out.
Seeds were then soaked in 70% ethanol for 3 minutes, rinsed three times with sterile distilled water, then immersed in a 1% (w/v) calcium hypochlorite solution containing two drops of tween 20 for 10 minutes. The seeds were then rinsed three times with sterile distilled water, then soaked in sterile distilled water to soften the inner shell. After 2 h of soaking, the seeds were stripped of their inner envelope and placed on the various germination media (Fig 1). All these operations were carried out in a laminar flow hood.

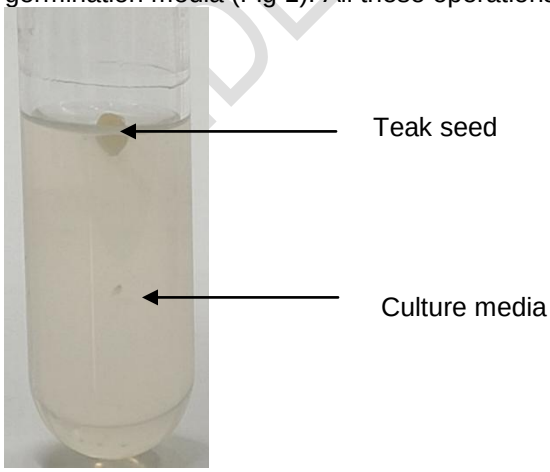

Fig 1: Seed placed on germination medium

2.2 Vegetative propagation of teak seedlings

After four weeks in culture, nodal segments and the shoot tip were excised from vitroplants under a laminar flow hood. These explants were placed on the same medium used for germination, to produce leafy shoots.

2.3 Rooting of leafy shoots

Whole and half strength McCowns Woody plant medium was used for rooting leafy shoots. These media were supplemented with 60 g/l of sucrose and different concentrations of AIB (0.5, 1, 2 and 4 mg/L). The pH was adjusted to 5.7 using NaOH (0.1 N) or HCL (0.1 N), then solidified with 8 g/l agar. The leafy shoots obtained previously and bearing four to five nodes were carefully taken and placed on the different rooting media.

2.4 Evaluation of in vitro germination of teak seeds

- Germination percentage:The germination percentage (GP) or germination capacity of the seeds for each medium is estimated by the following formula

$$\mathbf{PG} = \frac{\textbf{Number of seeds germinated}}{\textbf{Total number of seeds planted}} \mathbf{X\ 100}$$

- Average germination time:

The mean germination time (MGT) characterises the state of physiological maturity of the seeds. It is calculated using the formula:

$$\mathbf{TMG} = \frac{\sum \boldsymbol{n}.\ \boldsymbol{J}\mathbf{n}}{\boldsymbol{N}}$$

2.5 Growth of the in vitroplantlets

- Rate of normal seedlings:

The rate of normal seedlings corresponds to the ratio of the number of healthy seedlings obtained to the total number of seeds germinated after four weeks of cultivation.

$$\mathrm{TPN} = \frac{\text{Number of healthy seedlings}}{\text{Total number of germinated seeds}}$$

- Average number of leaves per seedling:

The average number of leaves per seedling (NMFP) was estimated by dividing the number of open leaves on each old seedling by the total number of seedlings after four weeks of cultivation.

$$\mathrm{NMFP} = \frac{\text{Total number of leaves on seedlings}}{\text{Total number of seedlings}}$$

- Averagenumber of nodes per seedling:

The average number of nodes per seedling (NMNP) was obtained by dividing the number of nodes on the teak seedlings obtained by the total number of nodes after four weeks of cultivation.

$$\text{NMNP} = \frac{\text{Number of seedling nodes}}{\text{Total number of seedlings}}$$

- Average length of the in vitroplantlets:

The average length of the shoots was obtained using a ruler graduated to the nearest millimeter. At the end of the fourth week of cultivation, the seedlings were measured from the collar to the tip of the young seedling. The average length of the seedlings (LMP) was determined by the ratio of the sum of the size of the seedlings to the total number of seedlings.

$$\text{MP} = \frac{\text{sum seedling size}}{\text{total number of seedlings}}$$

2.6 Growth of the developed leafy shoots

- Date of buds' induction:

The break date corresponds to the time elapsed between the date on which the explant was planted and the first induction of buds.

- Number of leafy shoots:

In this experiment, the average number of leafy shoots developed by each explant after four weeks of culture on the propagation medium was recorded.

- Average length of leafy shoots:

The length of the shoots was measured using a ruler graduated to the nearest millimeter. At the end of the eighth week of cultivation, the shoots were measured from the collar to the tip of the leafy shoot.

2.7 Rooting of the leafy shoots

After four weeks of cultivation, the date of appearance of the first root, the percentage of rooted shoots, the total number of roots and the length of the roots for each rooted micro cutting were recorded.

2.8 Statistical analysis

The experimental set-up used to study the influence of cytokinins and their concentrations on seed germination and teak bud induction is the complete randomized block.
Analysis of variance (ANOVA) was used to calculate the means of the various variables studied. These averages were classified by the Newman-Keuls test using STATISTICA 7.0 software at the 5% threshold.

RESULTS

1 Effect of cytokinin concentrations on teak seed germination

After five days in culture, seed germination was observed with the emergence and development of the radicle (Fig 2A). After one month of cultivation, the seedlings length were around 12 centimeters with two or three internodes (Fig 2B). On the other hand, some germinated seeds remained stunted and showed calluses at the level of their cotyledon on the culture medium (Fig 2 C).

Seed germination parameters are recorded in table 1. Cytokinin concentrations had no influence on the percentage and time of seed germination. The germination parameters considered in this study expressed statistically identical values whatever the cytokinin concentration in the germination medium. However, the concentrations of BAP and Kinetin into the germination medium had a significant influence on the percentage of normal seedlings. BAP at 0.5 mg/l gave the highest percentage of complete seedlings (83.46%), followed by kinetin at 0.5 mg/l (74.14). The lowest percentages of normal seedlings were obtained with TDZ (0.5 m /l).

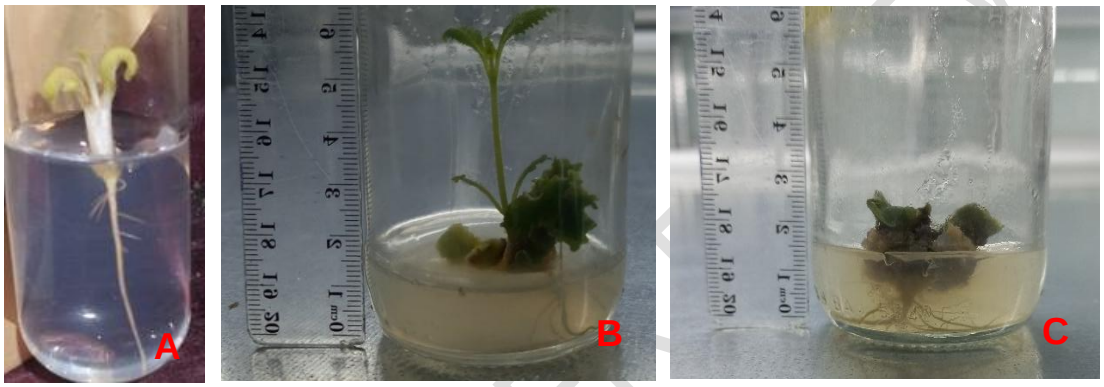

Fig2: Teak seedlings obtained by *in vitro* seed germination
A: radicle emergence, B: one-month-old normal seedling, C: undeveloped germinated seed.

Table 1. Germination parameters of teak seeds as a function of cytokinin concentrations

	Percentage of germination	Mean germination time (j)	Percentage of normal plantlets
Control *(MS0)*	78,97 ± 7,01	8,04 ± 1,05	55,25 ± 5,66^{cd}
B0,5	70,35 ± 5,52	7,36 ± 0,94	83,46 ± 8,500^{a}
B1	70,83 ± 8,61	7,65 ± 0,95	67,80 ± 6,417^{c}
B2	73,57 ± 9,741	7,67 ± 0,28	66,27 ± 7,81^{c}
B4	76,67 ± 6,34	7,94 ± 0,66	63,30 ± 8,48^{c}
K0,5	74,53 ± 6,61	7,20 ± 0,46	74,14 ± 7,833^{b}
K1	66,67 ± 8,76	7,28 ± 0,38	66,15 ± 9,57^{c}
K2	64,17 ± 5,20	7,78 ± 0,67	65,59 ± 3,47^{c}
K4	64,08 ± 7,42	8,32 ± 0,98	55,70 ± 8,02^{cd}
T0,01	63,61 ± 5,07	8,04 ± 0,40	54,96 ± 4,99^{cd}
T0,1	63,89 ± 7,46	8,10 ± 0,38	42,45 ± 8,65^{d}
T0,25	67,08 ± 9,00	8,25 ± 0,55	8,57 ± 3,14^{e}
T0,5	70,83 ± 6,27	8,39 ± 0,61	8,25 ± 2,29^{e}
P	*0,152*	*0,067*	*0,0001*

In the same column, means followed by the same letter are significantly identical at the 5% threshold (Newman-Keuls test). Mean ± standard deviation
MS0 : control medium; B0.5 to B4: medium containing 0.5, 1, 2 and 4 mg/l of BAP; K0.5 to K4: medium containing 0.5, 1, 2 and 4 mg/l of kinetin; T0.01 to T0.5: medium containing 0.01, 0.1, 0.25 and 0.5 mg/l of TDZ.

2Effect of cytokinin concentrations on the growth of teak seedlings

Various growth parameters were measured on seedlings obtained from seed germination. The values obtained in this experiment are given in Table 2. The concentrations of BAP and kinetin used in the germination medium had a significant effect on the size of the teak seedlings. Larger seedlings were obtained on medium containing 0.5 mg/l BAP (7.26 cm) and 0.5 mg/l kinetin (7.48 cm).
However, seedling size decreased with increasing concentrations of both hormones in the medium. In addition, all concentrations of TDZ reduced the size of teak seedlings.
As for the number of nodes and leaves of the seedlings, the highest values were expressed on the medium supplemented with 0.5 mg/l of kinetin.
As the concentrations of kinetin and BAP increased, the number of leaves of seedlings gradually decreased. The same observations were made with TDZ concentrations.

Table 2. Growth parameters of teak seedlings on culture medium containing different types of cytokinin

	Hight (cm)	**Number of nodes**	**Number of leaves**
Control *(MS0)*	$7,10 \pm 0,38^{b}$	$4,60 \pm 0,20^{b}$	$9,20 \pm 0,42^{b}$
B0,5	$7,26 \pm 0,73^{ab}$	$4,77 \pm 0,12^{b}$	$9,54 \pm 0,25^{b}$
B1	$6,92 \pm 0,56^{bc}$	$4,36 \pm 0,26^{bc}$	$8,72 \pm 0,60^{bc}$
B2	$6,32 \pm 0,41^{bc}$	$3,77 \pm 0,20^{bc}$	$7,54 \pm 0,35^{bc}$
B4	$5,52 \pm 0,55^{bc}$	$2,94 \pm 0,1^{bc}$	$5,88 \pm 0,47^{bc}$
K0,5	$7,48 \pm 0,40^{a}$	$5,99 \pm 0,15^{a}$	$11,98 \pm 0,32^{a}$
K1	$7,08 \pm 0,50^{b}$	$4,56 \pm 0,09^{b}$	$9,12 \pm 0,19^{b}$
K2	$6,39 \pm 0,42^{bc}$	$3,83 \pm 0,11^{bc}$	$7,66 \pm 0,22^{bc}$
K4	$5,86 \pm 0,40^{bc}$	$3,25 \pm 0,20^{bc}$	$6,50 \pm 0,37^{bc}$
T0,01	$6,21 \pm 0,56^{bc}$	$3,60 \pm 0,17^{bc}$	$7,20 \pm 0,37^{bc}$
T0,1	$4,67 \pm 0,28^{cd}$	$2,66 \pm 0,17^{cd}$	$5,32 \pm 0,36^{cd}$
T0,25	$4,54 \pm 0,07^{cd}$	$2,40 \pm 0,24^{cd}$	$4,80 \pm 0,80^{cd}$
T0,5	$4,18 \pm 0,32^{d}$	$2,20 \pm 0,20^{d}$	$4,40 \pm 0,40^{d}$
P	*0,0007*	*0,012*	*0,008*

In the same column, means followed by the same letter are significantly identical at the 5% threshold (Newman-Keuls test). Mean ± standard deviation, MS0: control medium; B0.5 to B4 medium containing 0.5, 1, 2 and 4 mg/l of BAP; K0.5 to K4 medium containing 0.5, 1, 2 and 4 mg/l of kinetin; T0.01 to T0.5: medium containing 0.01, 0.1, 0.25 and 0.5 mg/l of TDZ

3 Effect of cytokinin concentrations and the type of explants used on the induction of teak leaf shoots

Table 3 shows the effect of cytokinins on bud break and the growth of leafy shoots produced as a function of the type of explant used. The results showed that the type of explant and the

type of cytokinin had a significant influence on the bud break date. The shortest bud break date was obtained from shoot tip on medium supplemented with 0.5 mg/l BAP (7.43 days). In contrast, the longest bud break date for nodal segments was observed on medium supplemented with 2 mg/l BAP (12 days). In addition, explants (shoot tip and nodal segment) cultured on media supplemented with concentrations of TDZ greater than 0.01 mg/l had a very long bud break period. However, for this same cytokinin, the bud break date was higher for nodal segments. Generally, the bud break date is longer when the concentrations of the three hormones used increase in the culture medium.

The number of leafy shoots increased with the type of explants and cytokinin concentration used. The highest number of leafy shoots was obtained with nodal segments on medium supplemented with 2mg/L BAP (5 leafy shoots/explant) (Fig 3A).

The lowest numbers of leafy shoots were obtained on culture medium supplemented with TDZ (0.01, 0.1, 0.25 and 0.5 mg/l)(fig 3B).

As for the number of nodes, BAP at 2 mg/l expressed the highest value with the two types of explants, nodal segments and shoot tip respectively (8.33 and 7.25 nodes/explant)

The type of explants and the concentration of cytokinin added to the culture medium had a significant effect on the size of the leafy shoots obtained. The largest leafy shoot size was obtained with the shoot tips on medium supplemented with Kin at 2 to 4 mg/l (6 cm). The smallest leafy shoot size was obtained on the medium containing 0.5 mg/l Kin.

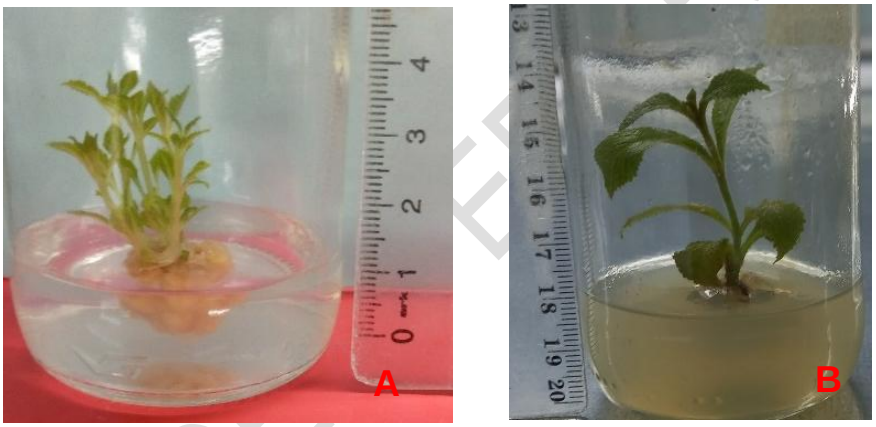

Fig 3: Leafy shoots from explant types
A: leafy shoot from nodal segment, **B**: leafy shoot from tip

Table 3: Effect of different cytokinins on the production of teak leafy shoots from various types of explants.

Explant type	Concentration	Date of bud break	Number of leafy shoots	Number of nodes	Number of leaves	Sizes
Tip	Control	10,75±1,60^{d}	1,28±0,49^{cd}	3,14±0,69^{c}	6,28±1,89^{c}	2,90±1,25^{f}
	B0,5	7,43±1,02^{g}	1,38±0,65^{cd}	4,00±3,56^{bc}	8,00±7,12^{bc}	3,28±2,11^{ef}
	B1	8,50±1,13^{f}	1,75±0,96^{bcd}	4,08±3,97^{bc}	8,15±7,93^{bc}	4,04±1,17^{e}
	B2	10,15±0,89^{de}	2,75±0,71^{bc}	7,25±2,55^{a}	14,50±5,01^{a}	4,10±2,04^{e}
	B4	10,60±1,43^{d}	1,40±0,55^{cd}	2,40±0,55^{d}	4,80±1,34^{d}	5,06±1,86^{c}
	K0,5	8,50±1,30^{f}	1,00±0,00^{d}	1,33±0,58^{e}	2,67±1,15^{e}	1,53±0,32^{h}
	K1	9,67±1,85^{e}	1,28±0,49^{cd}	2,00±1,15^{de}	4,00±2,31^{de}	3,01±2,58^{f}
	K2	11,67±1,85^{c}	1,33±0,52^{cd}	4,67±1,97^{bc}	9,33±3,93^{bc}	6,00±2,74^{a}

	K4	11,86±1,21^{bc}	1,67±0,58^{cd}	5,67±3,21^{b}	11,33±6,43^{b}	6,03±4,70^{a}
	T0,01	8,80±1,43^{f}	1,40±0,55^{cd}	3,00±2,16^{c}	6,00±3,58^{c}	3,93±1,97^{e}
	T0,1	10,67±1,85^{d}	1,25±0,50^{cd}	2,67±1,15^{cd}	5,34±1,67^{cd}	2,72±0,61^{fg}
	T0,25	11,75±1 ,60^{c}	1,00±0,00^{d}	2,40±1,67^{d}	4,80±2,31^{d}	2,60±1,36^{fg}
	T0,5	12,40±1,43^{b}	1,00±0,00^{d}	2,20±0,84^{d}	4,40±4,32^{d}	2,42±0,55^{g}
Nodal segment	Control	9,55±1,07^{ef}	2,22±0,44^{bcd}	3,55±1,81^{c}	7,11±4,12^{c}	3,22±2,62^{ef}
	B0,5	7,83±1,30^{fg}	2,28±0,49^{bcd}	3,11±1,76^{c}	6,22±3,39^{c}	3,30±1,14^{ef}
	B1	9,28±1,21^{ef}	2,33±0,50^{bcd}	4,00±2,38^{bc}	8,00±4,76^{bc}	4,24±2,24^{de}
	B2	12,00±1,07^{bc}	5,00±2,61^{a}	8,33±4,13^{a}	16,66±8,26^{a}	4,39±2,37^{de}
	B4	12,40±1,43^{b}	2,60±0,89^{bcd}	4,20±3,11^{bc}	8,40±6,23^{bc}	4,58±1,49^{d}
	K0,5	7,67±1,07^{fg}	3,12±2,03^{b}	2,86±2,85^{cd}	5,71±5,70^{cd}	4,56±4,78^{b}
	K1	9,50±1,01^{ef}	2,90±0,99^{bc}	3,89±1,45^{bc}	7,78±3,23^{bc}	4,66±3,60^{cd}
	K2	10,37±1,13^{de}	2,22±0,44^{bcd}	4,62±4,47^{bc}	9,25±8,94^{bc}	5,38±1,96^{bc}
	K4	11,14±1,21^{cd}	2,14±0,38^{bcd}	5,80±2,78^{b}	11,60±5,39^{b}	5,57±3,78^{b}
	T0,01	9,83±1,31^{e}	1,57±0,79^{bcd}	2,57±1,27^{cd}	5,14±1,57^{cd}	3,17±2,26^{f}
	T0,1	11,00±1,21^{cd}	1,28±0,49^{cd}	2,33±1,37^{d}	4,67±1,97^{d}	2,87±1,48^{fg}
	T0,25	12,67±1,30^{ab}	1,17±0,41	1,83±0,98^{de}	3,66±2,73^{de}	2,81±1,60^{fg}
	T0,5	12,71±1,21^{a}	1,00±0,00^{d}	1,57±0,79^{e}	3,14±2,52^{e}	1,90±0,66^{gh}
	P	*0,000*	*0,043*	*0,0008*	*0,0008*	*0,0008*

In the same column, means followed by the same letter are significantly identical at the 5% threshold (Newman-Keuls test). Mean ± standard deviation, MS0: control medium; B0.5 to B4 medium containing 0.5, 1, 2 and 4 mg/l of BAP; K0.5 to K4 medium containing 0.5, 1, 2 and 4 mg/l of kinetin; T0.01 to T0.5: medium containing 0.01, 0.1, 0.25 and 0.5 mg/l of TDZ

4 Effect of AIB concentration and type of medium on the rooting of leafy shoots

For this experiment, leafy shoots were excised and placed individually on different rooting media (Fig 4).

After four weeks of culture, the rooting of the explants was estimated using various parameters. The experimental results are shown in **Table 4.**

The type of growing medium and the concentrations of AIB had no significant effect on induction of the date of appearance of the first roots. On the other hand, a highly significant effect was recorded between hormone concentrations for the percentage rooting of leafy shoots.

The highest percentage of rooting of leafy shoots was obtained on 1/2 WPM supplemented with 4 mg/L AIB (72%). The lowest percentage was observed on 1/2 WPM supplemented with 0.5 mg/l AIB (42.86%). Additionally, for the same mineral salt content in the WPM medium, the rooting percentage increased progressively as the concentration of AIB increased. The number of roots per leafy shoot was identical on the culture medium whatever the AIB concentration.

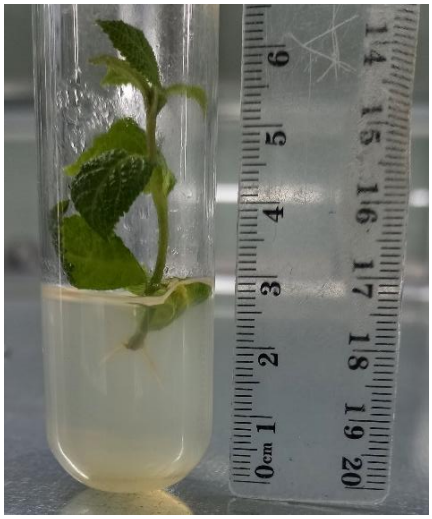

Fig 4: Leafy shoot on rooting medium

Table 4: Parameters evaluated during the rooting of leafy shoots on culture medium containing different concentrations of AIB

Type of medium	Concentration AIB	Date of appearance of 1st root	Percentage of rooted shoot	Total number of roots
WPM 1/2	AIB 0,5	16,13±7,83	42,86±0,00^{g}	2,13±1,36
	AIB 1	17,67±2,21	57,14±0,00^{f}	2,00±0,89
	AIB 2	13,50±2,78	63,64±0,00^{c}	1,75±0,46
	AIB 4	12,14±0,38	72,72±0,00^{a}	2,00±1,53
WPM 1	AIB 0,5	15,67±3,14	60,00±0,00^{e}	1,17±0,41
	AIB 1	14,33±0,52	60,00±0,00^{e}	1,67±1,03
	AIB 2	13,13±3,64	62,64±0,19^{d}	2,25±1,39
	AIB 4	12,00±0,00	65,45±0,00^{b}	3,20±1,64
	P	*0,688*	*P<0,05*	*0,114*

In the same column, means followed by the same letter are significantly identical at the 5% threshold (Newman-Keuls test). Mean ± standard deviation
AIB0.5 to AIB4 medium containing 0.5, 1, 2 and 4 mg/l of AIB; WPM0.5 to WPM1 medium McCowns Woody plant of proportion 0.5 and 1

4. Discussion

Teak seeds were germinated on MS medium supplemented with different concentrations of cytokinin. The results showed that adding cytokinin to the germination medium had no effect on the germination percentage of teak seeds. Cytokinins are not involved in the germination process. In addition, cytokinins do not inhibit teak seed germination. The hormone-free MS medium is sufficient for teak seed germination. This result confirms that of [6]. These authors obtained a germination percentage of 83% when they germinated teak seeds on MS medium without any growth regulators.

The teak seeds had a relatively short average germination time. This result could be explained by the fact that the water absorption phase, accompanied by stable oxygen consumption, followed by the resumption of respiration would have been carried out upstream and would have led to the rapid emergence of radicles. This result is in agreement with that of [10]on the in vitro germination of Moringaoleifera.

The lowest percentage of complete seedlings was obtained with TDZ concentrations. In fact, this cytokinin would prevent the circulation of endogenous auxin in the meristems of teak seedlings and which would strongly inhibit epicotyl growth. This result is consistent with that of [11]on the in vitro germination of teak. The latter described the stunted plantlets as hyperhydric seedlings.

The largest size and the highest numbers of nodes and leaves were observed in the medium supplemented with 0.5 mg/l kinetin. Low levels of kinetin in the culture medium are thought to promote cell elongation and leaf formation in young teak seedlings. In fact, this hormonal balance would favour exogenous gibberellin and auxin to carry out their activities on teak seedlings.

The shortest bud break date was observed with the shoot tips on the 0.5 mg/l BAP medium. This result could be explained by the fact that apical buds with a large quantity of endogenous BAP no longer require a significant supply of this hormone for the proliferation of leafy shoots. Similarly, the long bud break time of the nodal segment buds could be due to the high number of axillary buds present on the teak plant, which block the hormone before spreading to the internodes. This result is similar to that of [12], who obtained rapid bud break in the apical bud of a variety of Jatrophacurcas in contrast to other explants placed on the culture medium.Similarly, the work carried out by [13] on in vitro propagation of Jatropha from the shoot tips showed that budding took place after 7 days in culture.

The high number of leafy shoots was obtained with nodal segments on medium containing BAP at 2 mg/l. This concentration of BAP is optimal for triggering the formation of numerous buds from nodal segments. Thus, nodal segments, which are the site of several axillary buds, would favour the cellular metabolism of young leafy shoots when the BAP content becomes high in the culture medium, leading to a high proliferation of nodes and leaves in the latter. This result is similar to that of [14] on in vitro multiplication of Gmelinaarborea from nodal segments on BAP-containing medium. Also, our result corroborates that of [15].

It was shown in this study that 2 mg/l of BAP is required to induce bud neoformation.The largest size of leafy shoots was obtained from the shoot tips on the medium supplemented with Kin (2 to 4 mg/l). This result can be explained by the fact that the large quantity of kinetin promotes the activity of gibberellin on the growth of teak leafy shoots.

In vitro rooting depends on the concentration of auxin, the quantity of mineral elements and sugar in the culture medium. AIB is one of the auxins most widely used for rooting young teak leafy shoots, often in combination with NAA [16]. For this study, different concentrations of AIB were tested on the in vitro rooting of teak on McCown Woody plant medium containing 60 g/l sucrose. The incorporation of 4 mg/L of AIB into the culture medium significantly promoted root formation of leafy teak shoots.This result could be explained by the fact that a high concentration of AIB is a determining factor in teak rhizogenesis. In fact, this quantity of auxin is easily propagated throughout the root meristems for development into roots. This result confirms that obtained by**Etse*et al.* (2013)**[17] on Nauclealatifolia.

The reduction by half of the concentrations of mineral elements in the WPM medium containing a high concentration of sucrose was essential in the rhizogenesis of teak. Reducing the mineral concentration in the medium leads to a reduction in its nutrient resources. In addition, the large quantity of sugar in the culture medium would lead to osmotic and mineral stress in the explants. To cope with this stress, the leafy shoots emit roots in search of the nutrients they need to develop.**Jay-Allemand and Cornu (1986)**[18] showed that high concentrations of sucrose establish high osmotic pressures that can reduce the transport of water and nutrients from the base to the aerial part. In *Tectonagrandis*, the rooting rate was higher when explants were placed on 1/2 MS medium [19], [20].

5. CONCLUSION

This study showed that the incorporation of cytokinins into the culture medium had no influence on the percentage of *in vitro* germination of teak seeds. On the other hand, BAP and kinetin used at 0.5 mg/L in the culture medium were decisive for the production of normal teak seedlings. This investigation proved that it is possible to produce teak seedlings using the in vitro culture technique. For the two explants tested, the nodal segment was the best for mass production of leafy teak shoots. The BAP used at 2mg/L was the cytokinin that produced the highest number of leafy shoots. These leafy shoots had a better rooting rate when BAP at 4 mg/l was added to the culture medium made with 1/2 WPM and sucrose at 60 g/l. Future work will involve acclimatizing rooted teak vitroplants and then assessing the growth of plants transferred to a natural medium.

REFERENCES

1. Boucher D., Pipa E., Lininger K., Calen M.T., Sarah R. et Earl S. The root of the problem: what'sdriving tropical deforestationtoday? Cambridge, MA, USA: Union of ConcernedScientists/UCS_RootoftheProblem_DriversofDeforestation_FullReport.pdf, 2011. Report. pp 13-16
2. FAO et SEP-REDD+.Baseline forest data for REDD+ in Côte d'Ivoire. Mappingforestdynamicsfrom 1986 to 2015. Abidjan, IvoryCoast, 2017.
3. Kouakou Hervé Kouassi, Arthur Brice Konan-Waidhet, AffouéBerth Yao, Berenger Koffi, Ettibou Damase Kadjo. Simulation of the Dynamics of Land Cover and Land Use in the Lobo River WatershedUpstream of Nibéhibé (Center-West of Côte d'Ivoire).Journal of Geographic Information System, ,2020. Vol.12 No.5
4. Finifter.The timberindustry in Côte d'Ivoire. Policy Brief, 2010. 102 p
5. Ahanhanzo Corneille, Hubert Adoukonou-Sagbadja, Wilfred Yehouessi, Léonard Ahoton, Jean CossiGanglo, Micheline AgassounonDjikpo-Tchibozo, Arnaud Agbidinoukoun et Clément Agbangla. Study of the influence of mercuric chloride on the in vitro survival of explants and the ability to regenerate teak (Tectonagrandis L. f., Verbenaceae). Faculté des Sciences Agronomiques (FSA), Université d'Abomey-Calavi, Benin. Journal of Applied Biosciences, , 2013. 65 :4935 – 4944
6. Paulo C. P. F. J., Eduardo O. N. et Jonny E. S. P.*In vitro* establishment, germination and multiplication of teak Plant propagation: principles and practices,6th edition, Prentice Hall International inc., New-Jersey, USA, 2009. p.770
7. Emilio Mendoza De Gyves, Juwartina Ida Royani et EddoRugini. Efficient method of micropropagation and in vitro rooting of teak (Tectona grandis L.) focusing on large-scaleindustrial plantations. Biotech Center, Agency for The Assessment and Application of Technology, 2006

8. Sarra Arbaoui, Bruno Campanella, Roger Paul et TaoufikBettaieb. *In vitro*micropropagation of a fibre plant: kenaf (Hibiscus cannabinus L.). Département Agronomie et Biotechnologies Végétales, Institut National Agronomique of Tunisie. Nature et Technologie, 2013. p41 to 47.
9. Murashige T et Skoog F. A revised medium for rapidgrowth and bioassayswithtobacco tissue cultures. Physiol Plant, 1962. 15: 473-479.
10. SAIDI Nour El-yakine. Demonstration of an in vitro germination protocol for Moringaoleifera and propagation prospects. Dissertation, Université des Frères Mentouri Constantine 1 shallot (Allium cepa var. gr. aggregatum). Philipp J CropSci, 2020. 22 : 14-22
11. Muhammad AkrametFaheemAftab**.** Effect of Cytokinins on In vitro seed Germination and Changes in Chlorophyll and Soluble Protein Contents of Teak (Tectonagrandis L.Department of Botany, University of the Punjab, Lahore-54590, Pakistan, 2015
12. MedzaMve ; Mergeai. G ; Baudoin.J.-P. et Toussaint. A. Improving the in vitro multiplication rate of Jatrophacurcas L. TROPICULTURA, 2010. 28, 4, 200-204
13. Purkayastha J., Pugla T., Paul A., Solleti S.K., Mazumdar P., Basu A., Mohommad A., Ahmed Z. et Sahoo L. Efficient in vitro plant regeneration from shoot apices and gene transfer by particle bombardment in Jatrophacurcas. BiologiaPlantarum, 2010. 54, 1, 13-20.
14. Isidro S., Claudia A. et Kellen G.In vitro multiplication of GmelinaarboreaRoxb. Adulttrees. Revue. U.D.C.A. Act et Divcient, 2013. 16(1) : 97-102
15. MariappanSenthilkumar. An Improved in Vitro Micropropagation Technique for Teak (Tectonagrandis L.), International Journal of Advanced Biotechnology and Research (IJBR), PG and Research Department of Botany, Government Art's College, Dharmapuri Tamil Nadu, INDIA, 2015.- 636 705,
16. D'souza, L. et D'silva I. In vitro propagation of Anacardiumoccidentale L. plant, Cell Tissue and Organ Culture, 1992. 29(1) :1-6
17. EtseKodjoDjidjolé, RassimwaïPitekelabou et Atsou Vincent Aïdam. *In vitro*micropropagation and rhizogenesis in nauclea latifoliasmith (rubiaceae). Lomé, EuropeanScientific Journal, 2013. edition vol.9, No.24
18. Jay-Allemand C. and Cornu D. In vitro culture of isolatedembryos of commonwalnut (JuglansregiaL.). Annals Science Forest, 1986. 43 (2): 189-198.

19. Muhammad A. et Faheem A. Establishment of Embryogenic Cultures and Efficient Plant Regeneration System from Explants of Forced Softwood Shoots of Teak. Horticultural Plant Journal, 2016. 2 (5): 293-300.
20. Evandro.T.V., Rogalski M., Galeano E., Gilvano M.R., Brondani G.E., De Martin V.F., Da Silva L.A.& Carrer H. Efficient and new method for Tectonagrandis in vitro regeneration. Crop. Breed. Appl. Biot. 2017. 17 : 124-132.