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RESEARCH ARTICLE

IN VITRO REGENERATION OF JACKFRUIT (*ARTOCARPUS HETEROPHYLLUS* LAM., MORACEAE) IN CÔTE D'IVOIRE

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ABSTRACT

The Jackfruit is a forest species whose cultivation is totally unknown in Côte d'Ivoire. Its reproduction by seeds is not easy because the seeds are recalcitrant. Objective study is to develop a protocol for in vitro propagation of Jackfruit plants in Côte d'Ivoire in order to have quality and quantity plants. Terminal buds and microcuttings fragments from forest and garden nursery were aseptized and cultured. The impact of Calcium hypochlorite (Ca (OC12)) (2.5 to 7%) on aseptization of explants and the effect of Benzylaminopurine (BAP) (0.1 to 2 mg/L) on budding, Gibberellic Acid (GA3) (0.1 to 1.5 mg/L) on shoot development and Indole-3-Butyric Acid (IBA) (0.5 to 2 mg/L) on rhizogenesis were studied. Results show that with 3.5% Ca (OC12) for 10 minutes incubation, 80% of garden buds survived against 30% of forest bud. At, 2 mg/L BAP, 88% of bud sprout within 5 days of cultivation. with 1.5 mg/L of GA3, all shoots reached an average size of 2.5 cm in 30 days of cultivation. A 40% of shoots were rooted on 2 mg/L IBA. The different hormones used are conducive to the culture of this specie. Conditions thus defined in this work constitute a method for producing plantlets of Jackfruit a view to the propagation and popularization of this species in Côte d'Ivoire.

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INTRODUCTION

The Jackfruit (*Artocarpus heterophyllum* Lam.) is a forest species of the Moraceae family. It grows in both dry and cold climates in the same way as the breadfruit tree (Hossain and Haq, 2006). In India, Jackfruit is considered a noble wood. Its wood is ranked behind teak wood (*Tectona grandis*) for its durability and resistance to insects. These fruits, rich in calories, carbohydrates, proteins and vitamins, are processed for food (Eglal *et al.*, 2015). They are used in both immature and mature stages. Its pulp is sweet and tasty. It is used as a dessert. The bark and roots of Jackfruit tree are used to treat various ailments. Jackfruit trees are widely used in agro forestry and domestic farming systems (Hossain and Haq, 2006). It is cultivated not only for commercial purposes but also for reforestation, watershed protection and as a wind breaker (Falcao *et al.*, 2001).

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It is for this reason that our interest has been focused on the Jackfruit, which could be integrated as a reforestation plant in the vast reforestation program set up by the State of Côte d'Ivoire with the aim of increasing the forest area by 10% (MEFCI, 2017). Indeed, Côte d'Ivoire's forest cover, which was around 7.8 million hectares in 1990, has fallen to less than 2.5 million (Traoré K., 2018). However, Jackfruit is commonly reproduced by seed. Indeed, like most forest species, jackfruit seeds are recalcitrant, making the expression of germination power difficult. In addition, seed regeneration leads to a wide genetic diversity of offspring (Srinivasan *et al.*, 2012) and a long fruiting period of the trees (Samaddar, 1990). In response to these problems, several methods of vegetative propagation have been studied. Propagation by cuttings, layering and grafting of Jackfruit are not easy and limited techniques because of rooting difficulties and low rates of regrowth of grafts as in most forest trees. The difficulties encountered in natural and horticultural regeneration have led researchers to initiate the in vitro culture of jackfruit. This work has focused on micropropagation by culture of the nodal and apical segments (Rao *et al.*, 1981; Roy *et al.*, 1990, Amin and Jaiswal, 1993; Adiga *et al.*, 1998; Lee and Keng, 2005;

Ashrafuzzaman *et al.*, 2012; Jemal *et al.*, 2017) and on shoot rooting (khan *et al.*, 2010) and with . Whereas, the extension of Jackfruit cultivation in Côte d'Ivoire requires the development of an effective regeneration protocol. *In vitro* culture techniques could be used as a working tool to facilitate the rapid and compliant production of Jackfruit plants for their exploitation in the reforestation plan of Côte d'Ivoire. The objective of this study is to develop an efficient protocol for the *in vitro* propagation of Jackfruit in Côte d'Ivoire in order to have quality and quantity plants. The study will evaluate different concentrations of calcium hypochlorite and the time required for disinfection of explants and compare the different concentrations of Benzylaminopurine (BAP), Gibberellic Acid (GA₃) and Indole-3-Butyric Acid (IBA) in the constitution of culture media for budburst, growth and rooting of shoots.

METHODOLOGIE

Sampling and asepticization of plant material: Uninodal stem and internode fragments of about 1.5 cm were collected from 12-month-old forest and garden nursery young plants previously treated with Bio-Elit fungicide (0.12%) or not. The collected fragments were thoroughly washed with tap water. Then they were soaked in 70% alcohol for 1 minute and in calcium hypochlorite (Ca(OCl₂)) solutions at 2.5; 3.5 and 7% with two drops of tween 20 for 5, 10 and 15 minutes. The explants were then rinsed three times successively with sterile distilled water at an interval of 5 minutes each. This step will determine the best method of asepticization.

Preparation of culture media and culture conditions: Two types of media were used in this study. The bud break medium (MSD) prepared from macro and micro elements according to Murashige and Skoog (1962) to which were added the vitamin B5 complex (Gamborg *et al.*, 1968), 30 g/L sucrose and 6 g/L agar (Sigma, France). And, the rooting medium (MSR) prepared from the culture medium according to Murashige and Skoog (1962) diluted by half (MS/2), 30 g/L sucrose and 6 g/L agar (Sigma, France). Once the agar was added, the media were boiled to dissolve the gel and then the various hormones used were added. The pH media was adjusted to 5.8 with 1N NaOH or 1N HCl. The medium was dispensed into 30 mL jars and sterilized by autoclaving (Autester) for 20 min at 121 °C under 1 bar pressure. Seeding and subculturing were carried out under a horizontal laminar flow hood near the flame of a Bunsen burner. The jars seeded and sealed with parafilm were placed in a culture chamber maintained at a temperature of 25 ± 2 °C, under 2000 lux illumination provided by 60 cm long fluorescent tubes (Cool White Philips) under a 12 h photoperiod.

Cultivation of explants

Plantlets induction phase: The aseptic buds have been cleaned of stipules and dead parts in order to refresh the edges. The sanitized and refreshed explants were introduced one by one into jars containing 30 mL of induction medium. Five (5) types of induction media obtained from MSD base medium supplemented with 0.1; 0.5; 1; 1.5 and 2 mg/L of 6-benzylaminopurine (BAP) (Duchefa, Belgium) were used. A total of 30 explants were cultured on each of media. During 4 weeks of culture, the average bud break time of the explants, the percentage of healthy or contaminated buds, the average

number of new buds per shoot and the average number of leaves per shoot were determined in each culture medium.

Plantlets growth phase: The plantlets from the healthy cultures were transferred one by one into jars containing 30 mL of medium. The different elongation media consisted of MSD base medium supplemented with different concentrations of gibberellin acid (GA₃) at 0.1; 0.5; 1 and 1.5 mg/L. For each culture medium, 3 replicates of 20 shoots each, a total of 60 shoots per medium. The average size of the stems formed was measured after 4 weeks of culture.

Rooting phase: Plantlets with a length greater than or equal to 5 cm were cut off at the base with a sterile scalpel and transferred to jars on the basis of one plant per jar. Four (4) types of rooting medium (MSR) with 0.5; 1; 1.5 and 2 mg/L IBA were tested. A total of 30 shoots per medium was used. After 4 weeks of incubation, the percentage of leafy stems rooted and the average number of roots produced were recorded for each medium.

Statistical Analysis: The results obtained were processed by the Statistica 7.1 software using the one-criteria analysis of variance to test the difference between the different calculated rates. When a significant difference is revealed between the means for a given parameter. The LSD test at the 5% probability threshold was performed to compare the means.

RESULTS

Combined effect of the percentage of calcium hypochlorite (Ca(OCl₂)), the sterilization time and the origin of the explant on the plantlets induction

The interaction of different Ca(OCl₂) concentrations, the sterilization time and the origin of explant contributes to success of the cultures initiated. For stem fragments from plants without fungicide pre-treatment (Table 1); explant survival was 0% for all explants aseptized with 2.5% Ca(OCl₂). In contrast to the aseptic explants with 3.5% Ca(OCl₂) a survival rate of 50% for 15 mins of soaking for the explants from the nursery and 50% for 5-10 mins in the explants from the garden was obtained. These results indicate that 3.5% Ca(OCl₂) was effective for Jackfruit buds sterilization. Statistical analysis indicates that the concentration of Ca(OCl₂) and the origin of the plants significantly influenced the Jackfruit explants induction. For stem fragments from plants with fungicide pre-treatment (Table 1). A survival percentage of 80% at 10 minutes in garden plants aseptized with 3.5% Ca(OCl₂) was observed.

Effect of different concentrations of BAP on plantlets induction:

The addition of BAP to the culture media stimulates the budburst of shoots. The time to induction varies from 5 to 9 days (Table 2). After 4 weeks of culture, the culture media tested allowed shoot induction (Figure 1A-B). However, shoot formation was significantly influenced by the concentration of BAP (Table 2). Lateral bud formation around the main bud was also observed at the 1.5 and 2 mg/L BAP. At 2 mg/L BAP resulted in a maximum of 88% of explants each bearing 2.3 buds with an average size of 2.5 cm and an average number of 3.6 newly formed leaves (Figure 1C) (Table 2).

Table 1. Combined effect of the percentage of calcium hypochlorite (Ca(OCl₂)), the sterilization time and the origin of explant on the plantlets induction before and after fungicide treatment

Origin of explant	(Ca(OCl ₂)) (%)	Sterilization time (mns)	Explant before fungicide treatment			Explant after fungicide treatment	
			TC (%)	TS (%)	TN (%)	TC (%)	TS (%)
Forest nursery	2,5	5	100±0 ^c	0±0 ^a	0±0 ^a	100±0 ^b	0±0 ^a
		10	100±0 ^c	0±0 ^a	0±0 ^a	100±0 ^b	0±0 ^a
		15	100±0 ^c	0±0 ^a	0±0 ^a	100±0 ^b	0±0 ^a
	3,5	5	80±25 ^{de}	20±39 ^{ab}	0±0 ^a	80±40 ^b	20±54 ^a
		10	70±34 ^{cd}	30±43 ^{bc}	10±36 ^{ab}	80±40 ^b	20±53 ^a
		15	40±50 ^b	50±29 ^c	40±43 ^c	80±35 ^b	20±35 ^a
Garden nursery	7	5	0±0 ^a	100±0 ^d	100±0 ^d	-	-
		10	0±0 ^a	100±0 ^d	100±0 ^d	-	-
		15	0±0 ^a	100±0 ^d	100±0 ^d	-	-
	2,5	5	100±0 ^c	0±0 ^a	0±0 ^a	100±0 ^b	0±0 ^a
		10	100±0 ^c	0±0 ^a	0±0 ^a	100±0 ^b	0±0 ^a
		15	100±0 ^c	0±0 ^a	0±0 ^a	100±0 ^b	0±0 ^a
P(0,05)	3,5	5	60±43 ^{bcd}	50±48 ^c	0±0 ^a	40±25 ^a	60±36 ^b
		10	50±20 ^{bc}	50±49 ^c	0±0 ^a	20±53 ^a	80±47 ^b
		15	50±25 ^{bc}	40±38 ^{bc}	20±40 ^b	30±40 ^a	70±30 ^b
	7	5	0±0 ^a	100±0 ^d	100±0 ^d	-	-
		10	0±0 ^a	100±0 ^d	100±0 ^d	-	-
		15	0±0 ^a	100±0 ^d	100±0 ^d	-	-
	<i>P</i> ₁		0,43	0,30	0,20	0	0
	<i>P</i> ₂		0	0	0	0	0
	<i>P</i> ₃		0,28	0,45	0	0,78	0,73
	<i>P</i> ₄		0,66	0,90	0,69	0,78	0,73

± S : standard deviation ; within the same columns, values with the same letter are not significantly different according to LSD test ($P < 0,05$). TC : Contamination rate; TS : Survival rate; TN : Necrotic plants rate; p_1 : probability of the origin of explant; p_2 : probability of percentage of calcium hypochlorite; p_3 : probability of du Sterilization time; p_4 : probability of interaction

Table 2. Effect of different concentrations of BAP on bud induction of Jackfruit stem fragments after 4 weeks of culture

BAP (mg/L)	Average budding time (day)	Average number of buds induction (%)	Average number of induction plantlets/ explant	Average number of leaves formed per stem	Average stem size (cm)
0,1	9,5 ± 2,87 ^b	13	1 ± 0 ^a	0,6 ± 0,51 ^a	0,62 ± 0,31 ^a
0,5	9,8 ± 2,57 ^b	16	1 ± 0 ^a	0,8 ± 0,63 ^a	0,86 ± 0,21 ^{ab}
1	8 ± 2,26 ^b	31	1 ± 0 ^a	0,8 ± 0,63 ^a	1,02 ± 0,17 ^b
1,5	5,2 ± 1,68 ^a	67	2 ± 0,81 ^b	2 ± 1,24 ^b	1,37 ± 0,41 ^c
2	5 ± 0 ^a	88	2,3 ± 0,70 ^b	3,6 ± 0,96 ^c	2,5 ± 0,39 ^d
p	0,00	0,00	0,00	0,00	0,00

± S : standard deviation ; within the same columns, values with the same letter are not significantly different according to LSD test ($P < 0,05$).

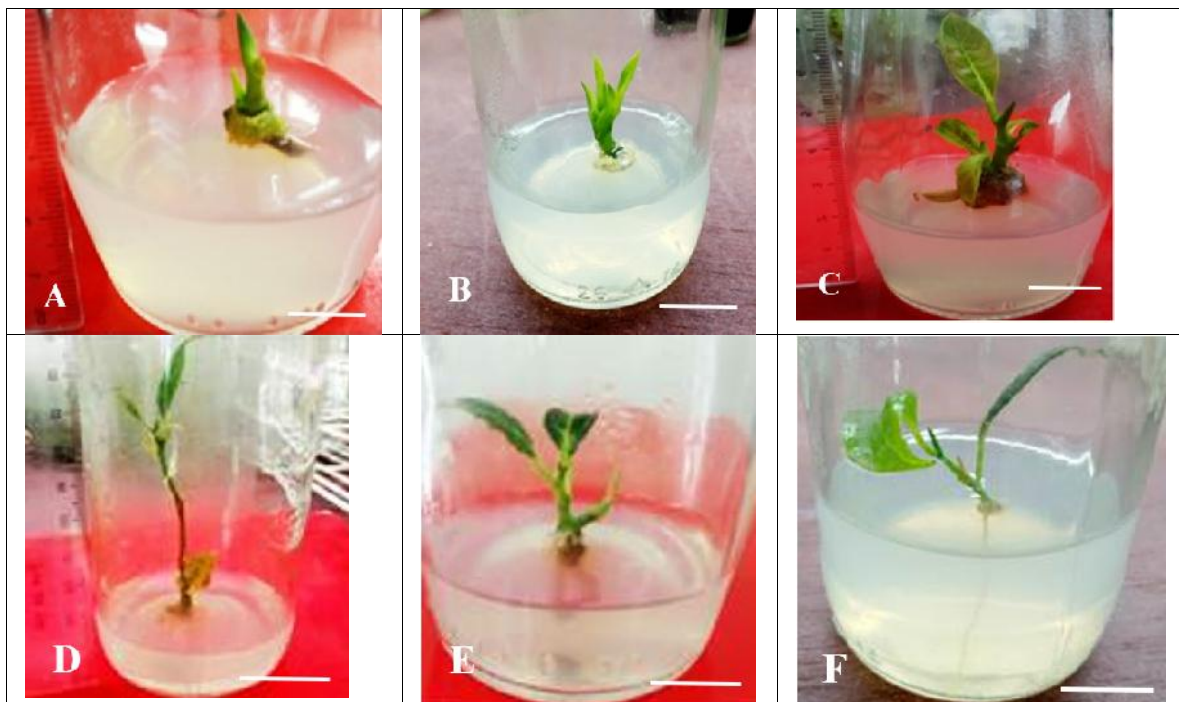


Figure 1: Different stages of in vitro regeneration of Jackfruit from uninodal stem fragments and terminal buds. (bar = 0,5 cm). A: Bud induction of an axillary bud on medium MSB5 + 2 mg/L BAP; B: Bud induction of a terminal bud on medium MSB5 + 2 mg/L BAP; C: Development and proliferation of an axillary bud on medium MSB5 + 2 mg/L BAP; D: Growth of an individualized shoot on medium MSB5 + 1,5 mg/L GA₃; E-F: rooting of an individualized shoot on medium 1/2 MS with 2 mg/L AIB

Table 3. Influence of GA₃ concentration on growth plantlets

GA ₃ (mg/L)	Average stem size (cm)	Elongation (%)
0,1	3,02 ± 0,11 ^a	0,52 ± 0,16 ^a
0,5	3,11 ± 0,52 ^{ab}	0,61 ± 0,18 ^{ab}
1	4,52 ± 0,12 ^b	2,02 ± 0,25 ^b
1,5	6,79 ± 0,31 ^c	4,29 ± 1,04 ^c
P	0,00	0,00

± S : standard deviation ; within the same columns, values with the same letter are not significantly different according to LSD test ($P < 0,05$).

Table 4. Influence of IBA concentration on rooting of Jackfruit shoots

AIB (mg/L)	Number of roots per shoot	Rooting rate (%)
0,5	0 ± 0 ^a	0 ± 0 ^a
1,0	0 ± 0 ^a	0 ± 0 ^a
1,5	0 ± 0 ^a	0 ± 0 ^a
2	1,16 ± 0 ^b	40 ± 43 ^b
p	0,00	0,00

± S : standard deviation ; within the same columns, values with the same letter are not significantly different according to LSD test ($P < 0,05$).

Effect of different concentrations of GA₃ on Jackfruit plantlets growth: Leafy stems transferred to the base medium MSB5 with 0.1 to 1.5 mg/L resulted in an increase in shoot size at the end of a subculture (Figure 1D). Leafy shoots showed an average elongation of 0.52 to 4.29 cm (Table 3). The culture medium with addition of 1.5 mg/L of GA₃ resulted in a 4.29 cm elongation of the plantlets, which was greater than that obtained with other culture media.

Influence of IBA concentration on rooting of Jackfruit shoots: Developed leafy stems induced weak rooting of their base (Figure 1 E-F) in the presence of culture media ½ MS enriched with 0.5 to 2 mg/L of IBA. After 4 weeks of culture, the maximum percentage of root differentiation by explants was 40% at 2 mg/L IBA and each had an average of 1.3 roots (Table 4).

DISCUSSION

The results obtained during the first disinfection phase showed that the rate of healthy buds and the rate of contamination vary significantly depending on the percentage of calcium hypochlorite, the sterilization time and the origin of the explant. Without treatment of the plants with fungicide, 2.5% calcium hypochlorite gave a high contamination rate (100%) in both types of explants. This high rate of contamination would be due to the low percentage of active chlorine in the sterilization solution which did not allow a good disinfection of the buds. This result could also be due to the very short incubation times. The active chlorine (2.5%) would not have had sufficient time to penetrate the bud tissues to eliminate the fungi or bacteria responsible for the infestation. These results are in contradiction with those of Khan *et al.* (2010) who reduced the contamination rate of their explants with a percentage of active chlorine between 0.5 and 1.25% after 15 minutes of incubation. The difference in results would be due to the fact that these authors worked with explants from greenhouses which reduced the infestation rate of these plants. Whereas, the explants used in this study came from a garden and the forest. These plants were therefore more exposed to fungi and bacteria. On the other hand, 3.5% calcium hypochlorite gave a high survival rate.

However, the survival rate was higher in garden plants than those collected from the forest. The low survival rate obtained in the forest plants would be due to the insufficient incubation time and the percentage of active chlorine (3.5%) to induce disinfection of these explants. On the other hand, the high survival rate recorded in garden plants would be due to the effectiveness of active chlorine (3.5%) for 10 minutes in these explants. This 10 minutes time was necessary for 3.5% active chlorine to penetrate the tissues of these explants to eliminate the agents responsible for bud infection. This result would also be related to the low exposure of the plants to fungi and bacteria as those of the forest. As for the 7% calcium hypochlorite, a higher rate of necrosis (100%) was obtained in both garden and forest plants. However, no contamination was observed. This result would be due to the very high percentage of active chlorine (7%) and the long incubation times. These buds being young, could not withstand these treatments. This resulted in the total destruction of the buds. Concerning the disinfection phase with fungicide treatment, the survival rates of the explants taken from the forest were not influenced by the different percentages of active chlorine (2.5% and 3.5%) used. The fungicide had no effect on the forest plants.

These results are consistent with the work of Rabah *et al.* (2007) who obtained a very low survival rate on explants collected in the forest. These authors showed that explants collected in the forest are much more exposed to microorganisms. Contrary to forest plants, explants from the garden gave a higher survival rate (80 %) at 10 mn, than those obtained from the latter before treatment. This result shows that the fungicide acted on the microorganisms in these explants allowing the active chlorine (3.5%) to eliminate a good number of bacteria and fungi at 10 mn incubation. The fungicide would have reduced the maximum amount of contaminants allowing the calcium hypochlorite to have a higher action on the microorganisms of the Jackfruit bud tissues in the garden. The fungicide would have eliminated surface bacteria and fungi. This allowed the chlorine to act directly on the microorganisms in the tissue. The fungicide used would have acted as a barrier for these explants.

The bud induction of terminal and nodal buds showed that BAP influenced the bud break rate of Jackfruit buds. The results are significantly different. The activating action of BAP on bud break was observed. Indeed, cytokinins are phytohormones responsible for the reduction of apical dominance, stimulation of cell division and axillary bud formation. However, low concentrations of BAP (0.1 to 1 mg/L) induced low bud break. And, the time of bud break varies between 8 and 9 days. The low BAP concentrations would have taken time to lift the apical dominance exerted by auxins, particularly AIA. Hence the long bud induction. This low concentration was not effective in stimulating cell division responsible for organogenesis. On the other hand, the concentration of 2 mg/L of BAP gave the best results. These results could be explained by the fact that this concentration was effective in inducing cell division in young Jackfruit buds within a short period of time. This would have favoured a rapid bud induction with a high number of large buds compared to the others. These results are consistent with those of Jemal *et al.* (2007) who obtained better results in Jackfruit bud proliferation with 2 mg/L BAP and Ashrafuzzaman *et al.* (2012). Thus, BAP at 2 mg/L is effective in activating cell division of young Jackfruit buds and inducing shoot

proliferation. The addition of GA₃ to the MSB5 medium significantly influenced the elongation of Jackfruit foliage shoots. These results are in agreement with the work of (Ndagijimana et al., 2014) which showed that gibberellin significantly favours internode elongation and accelerates the growth of the vitro culture. However, GA₃ at 1.5 mg/L was more effective on Jackfruit shoots. This concentration is optimal and would have allowed the gibberellin skeleton to activate the elongation of internode cells responsible for shoot length growth. These results show that the action of GA₃ depends on the concentration applied to the culture medium, taking into account the hormonal balance between the culture medium and the concentration of endogenous hormones and also the physiological state of each explant. Rooting difficulties are an obstacle to regeneration. The percentage of successful rooting requires IBA inducer treatment. Indeed, in *in vitro* culture the main function of auxins is the formation of callus and adventitious roots. When used in high concentrations, they induce calluses, and in low concentrations they induce roots (Matchakova et al., 2007). In this study, the maximum effect was observed at 2 mg/L with 40% of leaf shoots rooted. This is in contrast to the work of Khan et al. (2010) in Jackfruit where the maximum rooting percentage is 70% and that of Rahman and Blake (1988) in the same plant. This low rooting rate obtained in this study would be a function of the choice of auxin and working conditions. This rate, although low, remains considerable for this first *in vitro* investigation in Jackfruit in Côte d'Ivoire.

Conclusion

The jackfruit (*Artocarpus heterophyllus*. Lam) is a forest species native to India. It is considered a noble wood after teak (*Tectona grandis*). Jackfruit is used in the regeneration of degraded lands. The regeneration by seed which until then ensured the reproduction of the plants of the Jackfruit seems constraining with a long cycle of development. In this study, the development of an *in vitro* regeneration protocol for Jackfruit plants was undertaken. At the end of this study, the disinfection phase of the Jackfruit buds showed that calcium hypochlorite at 3.5% for 10 minutes soaking gave a healthy emergence rate of 50% to 80% with garden plants without or with fungicide treatment. The results obtained during this phase showed that the percentage of hypochlorite and the incubation time influenced the survival of the explants and especially conditioned the explants to induce shoots. This work shows that jackfruit can be propagated from fragments of uninodal stems. The results seem weak. Nevertheless, a protocol for the *in vitro* propagation of Jackfruit plants has been set up for the emergence of Jackfruit *in vitro* culture in Côte d'Ivoire. An in-depth study of the culture media and the appropriate hormonal regime of the different phases of *in vitro* propagation of Jackfruit and callus induction could be explored in our culture conditions in order to obtain plants with interesting traits for the extension of this species in Côte d'Ivoire.

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