World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

In vitro regeneration of cashew [*Anacardium occidentale* L. (Anacardiaceae)] trees from grafted plants

Bi Tra Jean-Innocent NANTI [1, *], Yah Gwladis GNAMIEN [1], Affoué Rachelle N'GUESSAN [1], Konan Marius KOUASSI [2] and Mongomaké KONÉ [2]

[1] *Laboratory for the Improvement of Agricultural Production, UFR Agroforestry, Jean Lorougnon Guédé University, BP 150 Daloa, Côte d'Ivoire.*
[2] *Laboratory of Biology and Improvement of Plant Production, UFR Natural Sciences, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire.*

World Journal of Advanced Research and Reviews, 2025, 27(01), 1593-1601

Publication history: Received on 13 May 2025; revised on 15 July 2025; accepted on 17 July 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.27.1.2563

Abstract

Propagation by seeds of the cashew tree (*Anacardium occidentale* L.) does not allow a faithful reproduction of the mother plant. The objective of this work is to establish an *in vitro* cashew tree regeneration protocol from the apex of grafted plants. To this end, apex explants taken from plants grafted in greenhouses were disinfected using sodium hypochlorite with 1.2 and 2.4% active chlorine and calcium hypochlorite 2.5 and 5% for 1; 2 ; 3 and 5 min, then cultured on MS medium supplemented with benzylaminopurine (BAP), kinetin and thidiazuron (TDZ) at various concentrations. The main results showed that disinfection of the explants with sodium hypochlorite at active chlorine 1.2 % for 2 min resulted in a low contamination rate (33%). The budding rate and the mean number of buds (1 to 2 buds per explant) did not vary while the best shoot growth (0.4 cm) were obtained on the medium without growth regulators. At the end of these experiments, the protocol for in vitro regeneration from the apex of the cashew tree was established.

Keywords: *Anacardium occidentale*; *In vitro*; Disinfection; Regeneration

1. Introduction

Native to Latin America, the cashew tree (*Anacardium occidentale* L.) was introduced to Côte d'Ivoire in 1951. It was only from 1959 to 1960 that cashew tree forest plantation programmes were carried out and extended to the entire ecologically favourable area, in particular the Sudano-Guinean savannahs [1]. The objective at the time was the protection of ecosystems seriously affected by deforestation and the fight against bush fires [2]. In 1990, the increase in the price of cashew nuts led to a craze for the cultivation of cashew trees (*Anacardium occidentale* L.) [3]. As a result, cashew trees appeared to producers as an alternative to the traditional cash crop of the north of the country, cotton, whose cultivation seemed threatened by falling global costs and major problems in the functioning of the sector [4].

The cashew tree is cultivated for the trade of its nuts. It is used as a raw material in the agri-food (chocolate, confectionery), pharmaceutical and cosmetic industries [5]. Cashew apples are also appreciated because of their richness in water (80 to 90%) and sugars (10 to 12%). This valorization of apple juice has been the subject of several studies [6,7].

It is estimated that between 1980 and 2020, the total area of land devoted to cashew cultivation increased from 526,250 hectares (ha) to 7,101,970 ha. As for world production, from 706,500 tons in 1990, it increased from 3.9 million tons in 2018 to more than 5,000,000 tons in 2022. This expansion has been observed mainly in Africa. Côte d'Ivoire has been

* Corresponding author: Bi Tra Jean-Innocent NANTI

the epicenter of this boom in global cashew production. This West African country is now the world's largest exporter of raw cashew nuts, with a turnover of $961 million in 2021 and a production of more than one million tons in 2022 [8].

This increase in national production is due to the increase in cultivated areas, which rose from 265,654 ha in 2000 to 1,350,000 ha in 2018 [9]. This galloping increase in cultivated land can lead to a lack or insufficiency of arable land and land disputes. Nut yields in Ivorian orchards remain low, in the range of 350 to 500 kg/ha, due to plantations created with unimproved plant material [10]. Conventional vegetative propagation techniques (layering, cuttings, grafting) have been used for the production of high-producing cashew planting material [11]. These methods allow the mother plant to be faithfully reproduced, but remain slow propagation tools [12]. In addition to these techniques, *in vitro* culture methods of plant tissues and organs have been used [13; 14]. Biotechnology is a powerful tool for overcoming natural barriers, and thus represents an alternative and complementary approach to conventional breeding efforts [15]. Unfortunately, the micropropagation of woody species from explants taken from mature trees is strongly hampered by serious constraints such as the difficulty of disinfection and browning of the explants and the low rate of bud induction, thus making their multiplication difficult *in vitro*. Despite these constraints, authors have reported the production of cashew nut plant material through micropropagation [16; 13]. In addition, the conditions for growing these explants are not clearly established. The objective of this study is to establish a regeneration protocol for the production of cashew nut vitroplants from grafted plants.

2. Materials and methods

2.1. Plant material

The plant material consists of young cashew plants three months old obtained by grafting (Figure 29). The grafts were taken from high-yielding trees in an industrial plantation in Farako, Bogofa Sub-Prefecture, Gontougou region (Côte d'Ivoire). Rootstocks are obtained by germinating mature nuts harvested in Gohitafla, in the Marahoué region (Côte d'Ivoire).

Figure 1 Three-month-old grafted cashew tree plant

2.2. Preparation of culture media

The base medium of Murashige and Skoog [17] was used for this study. To this medium, 30 g/l sucrose and an antioxidant (activated charcoal 0.2%) and various concentrations of cytokinins were added. The pH was adjusted to 5.8 using a pH meter with HCl (1N) and/or NaOH (1N). The gelling agent, phytagel (3g/l) has been added. The resulting solution was stirred for 10 min and then brought to a boil in order to solubilize the phytagel. A volume of 30 ml of this

medium was dispensed into pots which have been hermetically sealed with caps. These media were sterilized at autoclave at 121 °C for 20 min under a pressure of 1 bar and then transferred to a room of culture after cooling.

2.3. Preparation and disinfection of explants

Grafted plants treated with fungicide once a week were used as a source of explants. The twigs taken early in the morning from these plants were soaked in soapy water for 30 minutes and then rinsed thoroughly with tap water. Under the laminar flow hood, these twigs are immersed in 70% alcohol for 1 min, then in various concentrations of sodium or calcium hypochlorite containing 2 drops of tween 20 at different times.

2.4. Cultivation of explants

After the disinfection phase, the apex explants were taken from the disinfected twigs and placed on the MS medium supplemented with 0.2% activated charcoal and various concentrations of cytokinins

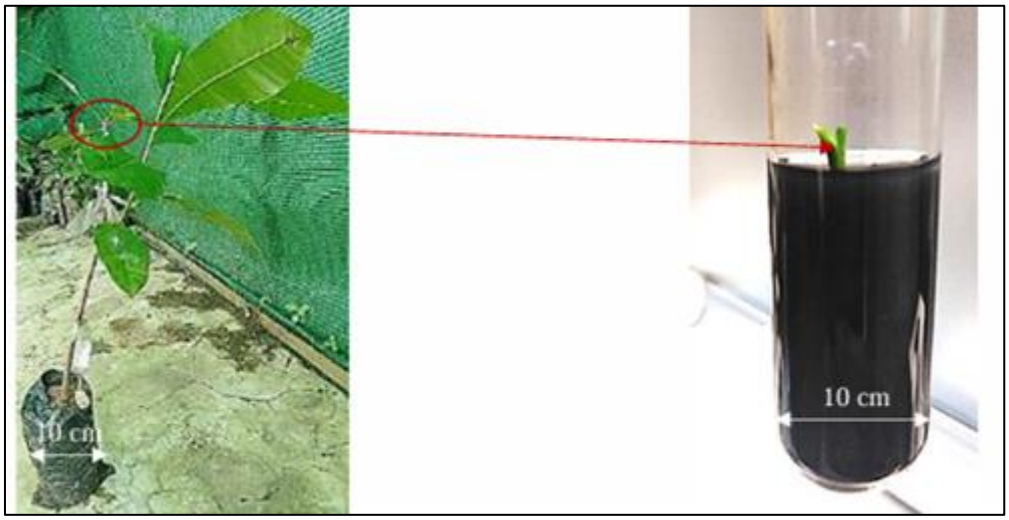

Figure 2 Culturing the apex

2.5. Effect of disinfecting agents on explant contamination and viability

Sodium hypochlorite at 1.2 and 2.4% active chlorine and calcium hypochlorite 2.5 and 5% were the disinfecting agents used. In these solutions, the explants were soaked at 1; 2 ; 3 and 5 min. At the end of the immersion time, the explants were removed and rinsed three times with sterile distilled water. The viability of the explants was evaluated after three weeks of cultivation. The experiment was repeated three times at a rate of 10 replications per treatment and per repeat.

2.6. Effect of cytokinins on cashew bud induction

The apex explants were cultured on MS medium supplemented with three cytokinins at different concentrations. Thus, benzylaminopurine (BAP) and kinetin at concentrations of 0, 1, 2, 4 and 6 mg/L and thidiazuron (TDZ) at concentrations of 0; 0.001, 0.01, 0.1 and 1 mg/L were used as growth regulators. The apex explants were placed on these different media. The experiment was repeated three times for each medium, at a rate of 10 replications per repeat

2.7. Experimental design and data analysis

The experiments were carried out in a completely randomized design with ten replicates and each individual treatment was repeated three times. Bud frequency, number of buds per explant were submitted to analysis of variance (ANOVA) to detect significant differences between means of each growth regulator and disinfectant. Means differing significantly were compared using Newman-Keuls multiple range test at.

3. Results

3.1. Effect of disinfectants and their concentrations and duration of disinfection on explant contamination

The results regarding the effect of disinfectants are presented in table I. Analysis of the results in this table showed a very significant effect of disinfecting agents on the contamination rate ($P < 0.001$). This is because this contamination rate is higher with calcium hypochlorite compared to sodium hypochlorite, regardless of the duration of disinfection. In addition, this rate decreases with increasing disinfection time. Thus, the highest contamination rates are 87.09% and 90% respectively at 1 min and 2 min with sodium hypochlorite (1.2% active chlorine) and calcium hypochlorite (2.5%).

Table 1 Contamination rate of cashew tree explants after disinfection with sodium and calcium hypochlorite

Disinfectants	Concentrations (%)	Duration (min)	Contamination rate
Sodium hypochlorite	1.2	1	87.09 ± 2,9 a
		2	33 ± 8.05 cd
		3	30 ± 7.87 cd
		5	18 ± 6.68 d
	2.4	1	78 ± 5.68 a
		2	18 ± 6.68 d
		3	24 ± 7.39 d
		5	12 ± 5.68 d
Calcium hypochlorite	2.5	1	90 ± 00 a
		2	90 ± 00 a
		3	72 ± 6.68 ab
		5	63 ± 7.65 ab
	5	1	72 ± 6.68 ab
		2	51 ± 8.28 ab
		3	66 ± 7.39 ab
		5	33 ± 8.05 cd
Probability	P1		< 0.001
	P2		< 0.001
	P3		< 0.001
	P4		0.47

In the same column, the means followed by the same letter are significantly identical to the 5% threshold (Student Newman-Keuls test). Mean ± standard error (P1): probability related to the nature of the disinfectant agent; (P2): probability related to the concentrations of disinfectants; (P3): probability related to the duration of disinfection; (P4): probability of the disinfectant agent-concentration-duration of disinfection interaction.

3.2. Effect of disinfecting agents on the viability of the explant

After one month of culture of the apexes, the rate of explants that survived the disinfectants is shown in table 2. Analysis of this table reveals that the disinfecting agent did not have a significant effect on explant viability ($P > 0.05$). In contrast, concentrations and duration of disinfection had a very significant effect on explant survival ($P < 0.001$). These concentrations ranged from 18% obtained with sodium hypochlorite at 2.4% active chlorine during 5 min of disinfection, to 87% obtained with sodium hypochlorite at 1.2% active chlorine during 1 min of disinfection. Taking into

account the contamination rate and survival rate, sodium hypochlorite with 1.2% active chlorine used for 2 min was the most indicated for effective disinfection of the apex explants of the cashew tree.

Table 2 Cashew apex survival rate after disinfection with sodium calcium hypochlorite

Disinfectants	**Concentrations (%)**	**Duration (min)**	**Viability rate**
Sodium hypochlorite	1.2	1	87.09 ± 2.9 a
		2	81 ± 5.01 a
		3	48 ± 8.34 bcd
		5	33 ± 8.05 cd
	2.4	1	66 ± 7.39 ab
		2	51 ± 8.28 bc
		3	30 ± 7.88 cd
		5	18 ± 6.68 de
Calcium hypochlorite	2.5	1	83.79 ± 4.31 a
		2	66 ± 7.39 ab
		3	48 ± 8.34 bc
		5	48 ± 8.34 bc
	5	1	72 ± 6.68 ab
		2	45 ± 8.36 bcd
		3	42 ± 8,34 bcd
		5	6,33 ± 4,17 e
Probability	P1		0,916
	P2		< 0.001
	P3		< 0.001
	P4		0,171
In the same column, the means followed by the same letter are significantly identical to the 5% threshold (Student Newman-Keuls test). Mean ± standard error (P1): probability related to the nature of the disinfectant agent; (P2): probability related to the concentrations of disinfectants; (P3): probability related to the duration of disinfection; (P4): probability of the disinfectant agent-concentration-duration of disinfection interaction.			

3.3. Effect of cytokinins on cashew budding

After a month of cultivation, the cultivated apexes have budded. These buds turned into leafy shoots after six months of cultivation (Figure 3). Values for the effect of bensylaminopurine, kinetin and thidiazuron on budding have been reported in Table 3. Analysis of the results shows that the cytokinins used have a similar effect on budding time, budding rate, number of buds and mean shoot size ($P > 0.05$). However, cytokinin concentration had a significant influence on budding time and mean bud count ($P < 0.05$) and a very significant effect on mean bud size ($P < 0.001$). No cytokinin-concentration interaction effect was observed on the various parameters observed ($P > 0.05$). The first bud appears eight days at most after the cultivation of the explants; The budding rate ranged from 39% to 61%. In addition, the average number of buds per explant did not exceed 2. Regarding the average size of the shoots, it varied from 0.28 cm on medium supplemented with TDZ 1mg/L to 0.42 cm on medium lacking cytokinin.

Table 3 Parameters of budding of cashew apex according to cytokinins and their concentrations

Cytokinins	Concentrations (mg/L)	Budding time (day)	Budding rate (%)	buds number	Average height
BAP	1	6.87 ± 0.17 a	42 ± 8.33 a	1.4 ± 0.09 a	0.42 ± 0.02 a
	2	7.3 ± 0.19 a	54 ± 8.18 a	1.6 ± 0.1 a	0.40 ± 0.01 ab
	4	7.3 ± 0.23 a	60 ± 7.87 a	1.6 0.09 a	0.37 ± 0.01 abc
	6	7.43 ± 0.18 a	48 ± 8.33 a	1.5 ± 0.09 a	0.31 ± 0.01 cd
KIN	1	6.97 ±0.18 a	45 ± 8.35 a	1.57 ± 0.1 a	0.4 ± 0.02 ab
	2	6.9 ±0.22 a	54 ± 8.1 a	1.5 ±0.09 a	0.37 ± 0.01 abc
	4	7.37 ± 0.25 a	54 ± 8.18 a	1.43 ± 0.09 a	0.38 ± 0.01 abc
	6	7.6 ± 0.22 a	54 ±8.18 a	1.4 ±0.09 a	0.31 ± 0.1 cd
TDZ	0.001	7.4 ± 0.20 a	60 ± 7.87 a	1.57 ± 0.12 a	0.39 ± 0.01 abc
	0.01	7.33 ± 0.19 a	66 ± 7.39 a	1.77 ± 0.12 a	0.37 ± 0.01 abc
	0.1	7.63 ± 0,17 a	54 ± 8.18 a	1.73 ± 0.11 a	0.35 ± 0.01 abc
	1	7,67 ±0.18 a	57 ± 8.05 a	1.57 ± 0.10 a	0.28 ± 0.01 d
Probability	P1	0.059	0.597	0.050	0.334
	P2	0.003	0.142	0.037	< 0.001
	P3	0.828	0.905	0.848	0.965

In the same column, the means followed by the same letter are significantly identical to the 5% threshold (Student Newman-Keuls test). Mean ± standard error (P1): cytokinin-related probability; (P2): probability related to cytokinin concentrations; (P3): probability of cytokinin-BAP concentrations: Benzylaminopurine; Kin: Kinetine; TDZ: Thidiazuron

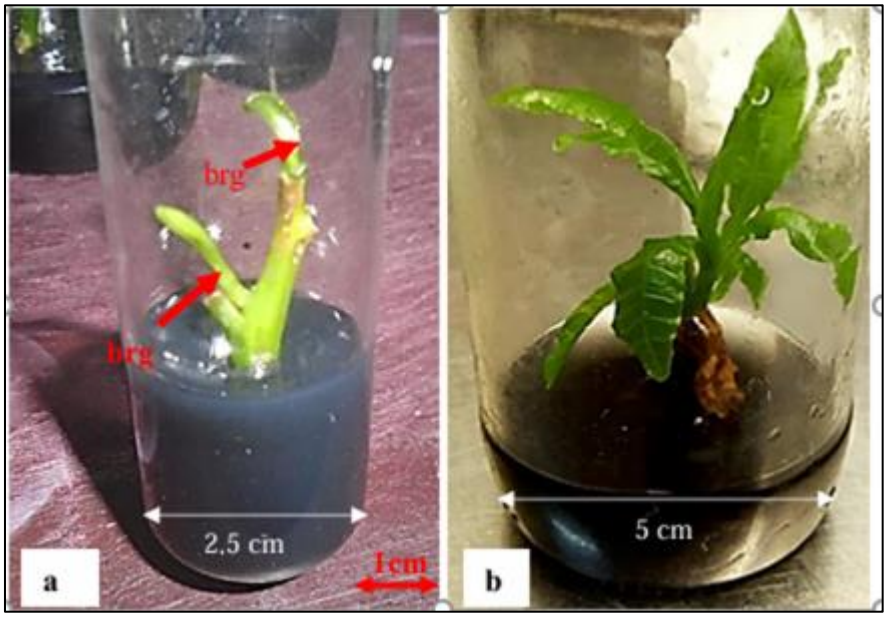

Figure 3 Bud induction and leafy shoot development from the explant apex taken from grafted plants(a) bud induction; (b) bud development into leafy shoots (brg): buds

4. Discussion

Exogenous and endogenous contamination of explants has always considerably limited the in vitro culture of plants. Microorganisms, including bacteria and fungi, as well as some mites that carry them, can be associated with explants used for in vitro plant regeneration [18]. Thus, one of the possible reasons for infection of explants could be the origin of the plant material used. [19] suggested that mother plants used for plant tissue cultures should be grown under controlled conditions (in greenhouses or growth chambers) to minimize the risk of microbial contamination of organs. However, regardless of the origin of the explants, the use of good disinfection techniques, depending on the plant organ or tissue considered, should allow the elimination of microbial contamination [20]. The losses observed vary depending on the nature of the disinfecting agent, the concentration and the duration of disinfection. In this study, two chemical solutions were tested for their ability to neutralize microorganisms that infect cashew tree explants

The largest number of disinfected explants, both of which survived the effect of the chemical agents, was obtained after a 2-minute soak in sodium hypochlorite with 1.2% active chlorine. This high survival rate with this disinfecting agent attests that sodium hypochlorite disinfection is effective for the surface sterilization of the apex explants of the cashew tree. The effectiveness of sodium hypochlorite makes it the most widely used disinfectant [21; 22] for in vitro initiation of explants, compared to calcium hypochlorite. [23] showed that sodium hypochlorite used at 3.6% active chlorine is effective in disinfecting *Lippia multiflora* explants. Indeed, sodium hypochlorite seems to penetrate inside cells and therefore acts more effectively on endogenous pathogens in cells. In addition, sodium hypochlorite, due to its dual antifungal and antibacterial action, has been shown to significantly reduce fungal and bacterial infections [24].

After explants disinfection phase, the study of the influence of cytokinins on budding showed that the number of buds per explant varied between 1 and 2. [25] showed the low potential for induction of the apex bud compared to nodal segments. The production of a bud by *in vitro* culture from cashew explants from an *ex vitro* source was reported by [26], after two months of culture. This author obtained a single bud per apex explant on the MS and LS media. The low organogenic potential of the apex was reported by [27]. These researchers obtained a maximum of two buds per explant (apex) when they worked on the micropropagation of cashew nuts. [28] did not observe any buds on the apex of the explant collected from plants obtained by ex vitro germination. However, other authors have reported obtaining a greater number of buds in *in vitro* culture of the apex of the cashew nut. [29] observed two to three axillary shoots per explant during cashew micropropagation, while [30] obtained three to four buds per explant. The low number of buds per explant obtained in this study compared to some previous work may be due to the genotype of the plants used as a source of explants. Indeed, most plants exhibit species-specific genotypic regeneration. Within the same species, one genotype produces buds while another can only produce embryos [31]. However, several authors mention that only certain genotypes seem to have a high organogenic power. In many species, this ability appears to be controlled by genotype [32]. Such control of regeneration has been reported in legumes, particularly in *alfalfa* by [33], in Trifolium repens L. by [34] and many other species. [35] indicate the presence of a few major genes that can control organogenesis in peas. Similarly, [36] succeeded in significantly increasing the embryogenic frequency in *Médicago sativa* L. after two stages of recurrent selection. [37] found in a diploid alfalfa genotype that bud differentiation from callus is controlled by two dominant genes called Rn1 and Rn2, whose simultaneous presence allows for a high regeneration rate (more than 75% of explants). The budding rate is about 60%. This result corroborates those of [13] who obtained a budding rate of 57.2% in *in vitro* cashew culture from explants taken from plants grown in greenhouses. Several other authors have obtained 80-100% budding in the same species with high concentrations of sodium hypochlorite [38; 25]. The low budding rates recorded in this study could be explained by the fact that chemical solutions used at low concentrations have difficulty penetrating the explants. These are unable to neutralize the microorganisms that have infected the interior of the plant material [22]. The disinfectant power of sodium hypochlorite depends essentially on the active chlorine content. However, a very high percentage of chlorine in a sodium hypochlorite solution would be lethal to microcuts.

5. Conclusion

The disinfection techniques used have shown that cashew tree explants require surface disinfection. This can be done by soaking the explants in sodium hypochlorite with 1.2% active chlorine for 2 min. The established protocol allowed the induction of one to two buds per apex explant from grafted cashew plants.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Goujon P., Lefebvre A., Leturcq Ph., Marcellesi A.P. & Praloran J.C. Studies on the cashew tree. Fruits, 28 (3): 217-225, 1973.

[2] Gouma M. Analysis of WTO negotiations on agriculture for the formation of an appropriate policy for the development of the cashew sector in Ivory Coast, Professional thesis, University of Cocody, Abidjan, 46p, 2003.

[3] Nugawela P., Baldé A. & Poublanc C. The Cashew Value Chain in Senegal. Analysis and Strategic Framework for Growth in the Sector. USAID/Economic Growth Program, 78p, 2006.

[4] Ricau P. & Konan C. The cashew industry in Côte d'Ivoire: stakeholders and organization. Mission report, March-July 2010, 43p, 2010.

[5] Aliyu O.M Pollen-style compatibility in cashew (*Anacardium occidentale* L.). Euphytica, 158: 249-260, 2007.

[6] Dornier M., Abreu F., Perez A.M. & Reynes M. Potential of tangential microfiltration on mineral membranes for the clarification of cashew apple juice. Fruits, 60: 33-40, 2005.

[7] Gökmen V. & Cetinkaya Ö. Effect of pretreatment with gelatine and bentonite on permeate flux and fouling layer resistance during apple juice ultrafiltration. Journal of Food Engineering, 80: 300-305, 2007.

[8] Aboa A. Slump in global cashew demand pushes Ivory Coast industry to verge of collapse, Reuters ,2023.

[9] FIRCA The Progress Sector, Information Magazine of the Interprofessional Fund for Agricultural Research and Advice. Magazine, Abidjan, Ivory Coast, 55p, 2018.

[10] Aliyu O.M. & Awopetu J.A. Assessment of genetic diversity in three populations of cashew (*Anacardium occidentale* L.) using protein-isoenzy, 2007.

[11] Boutherin D. &. Bron G. Propagation of horticultural plants. Technical editions and documentation. Lavoisier, Paris, 20p, 1989.

[12] Aliyu O.M. Application of tissue culture to cashew (*Anacardium occidentale* L.) breeding: An appraisal. African Journal of Biotechnology, 4 (13): 1485-1489, 2005.

[13] Sija S.L., Potty V.P. & Santhoshlal P.S. In Vitro Shoot Proliferation from Excised Shoot Tip and Nodal Segment of *Anacardium occidentale* L. International Journal of Current Microbiology Applied Science, 5 (3): 635-642, 2016.

[14] Nanti B.T.J-I., Kimou S.H., Gnamien Y.G., KOUASSI K.M. and KONÉ M. *In vitro* culture of cashew (*Anacardium occidentale* L.): influence of disinfection, antioxidants and carbon source on germination and growth. Int. J. Biol. Chem. Sci. 17(6): 2464-2474, 2023.

[15] Puonti-kaerlas J., Stabel P. & Eriksson T. Transformation of Pea (*Pisum sativum* L.) by *Agrobacterium tumefasciens*. Plant Cell Report, 8: 321-324, 1989.

[16] Kamshananthi T. & Thayamini H.S. Induction of somatic embryogenesis from cotyledon explants of cashew (*Anancardium occidentale* L.). Journal of Agricultural Technology, 8 (6): 2089-2099, 2012.

[17] Murashige T. & Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiology Plant, 15: 473-497, 1962.

[18] Omamor I.B., Asemota A.O., Eke C.R. & Eziashi E.I. Fungal contaminants of the oil palm tissue culture in Nigerian Institute for Oil Palm Research (NIFOR). African Journal of Agricultural Research, 2 (10): 534-537, 2007.

[19] Leifert. C. & Waites W.M. Dealing with microbial contaminants in plant tissue and celI culture : hazard analysis and critical control points. In Lumsden P.J., Nicholar J.R. et Davies W. J. editions, Physiology, Growth and Development of Plants in culture, KIuwa Academic Publishen (Netherlands), pp: 363-378, 1994.

[20] Naveena V. & Joy P.P. Plant tissue culture contaminants identification and its response to fumigation. Technical Report, January 2016, 10p, 2016.

[21] Boxus P., Damiano C. & Brasseur E. Strawberry. In ammirato P.V., Evans D.A., Sharp W.R., Yamada Y - handbook of plant cell culture, vol. 3, New York: Macmillan, pp: 453-486, 1984.

[22] López-aranda J.M., Pliego-alfaro F., López-navidad I. & Barcelómuñoz M. Micropropagation of strawberry (Fragaria x ananassa duch.). Effect of mineral salts, benzyladenine levels and number of subcultures on the *in vitro* and field behaviour of the obtained microplants and the fruiting capacity of their progeny. Journal of. horticultural. science, pp: 625-637, 1994.

[23] Soumahoro A.B., Koné T., Koné M., Konaté S., Kouadio J.Y. & Zouzou M. Establishment of an effective germination protocol for savannah tea seeds (*Lippia multiflora* MOLD.). African Agronomy, 26 (2): 137-145, 2014.

[24] Badou T.B., Pita S.J., Houedjissin S.S. & Ahanhanzo C. Control of endogenous germs by the use of antimicrobial agents and response of different explants during the *in vitro* initiation phase of pineapple (*Ananas comosus* (L.) Mill var. comosus). Journal of Applied Biosciences, 121: 12129-12143, 2018.

[25] Thimmappaiah, Shirly R.S. & Sadhana P.H. In vitro propagation of cashew from young trees. *In vitro* Cellular and Developmental Biology Plant, 38: 152-156, 2002.

[26] Hedge M. Ph.D. thesis, Tamil Nadu Agricultural University, Coimbatore, India, 169p, 1988.

[27] Sudripta D., Timir B., Jha & Sumita J. *In vitro* propagation of cashewnut. Plant Cell Reports, 15: 615-619, 1996.

[28] Lakshmi-Sita G. Differentiation of embryos and leafy shoots from callus cultures of cashew (*Anacardium occidentale* L.). Abstracts XIII Plant Tissue Culture Conference, 18-20 October. Shillong, India. Abstract No. 71, 1989.

[29] Lievens C., Pylyser M. & Boxus P. Clonal propagation of *Anacardium occidentale* L. by tissue culture. Fruits, 44: 553-557 1989.

[30] Keshavachandran R. & Khader A.M.D. Micropropagation of cashew (*Anacardium occidentale* L.). Journal of Plantation Crops, 20: 299-300, 1990.

[31] Auge R., Beauchesne G., Boccon-Gibod J., Decourtye L., Digat B., Jalouzot R., Minier R., Morand J-Cl., Reynoird J.P. & Strullu D.G. *In vitro* culture and its horticultural applications. 3rd revised and expanded edition. Ed. Tec et Doc. Lavoisier, 225p, 1989.

[32] Dodeman V. L., Ducreux G., & Kreis M. Zygotic embryogenesis versus somatic embryogenesis. Journal of Experimental Botany, 48 (8): 1493-1509, 1997.

[33] Brown D.C.W. & Atanassov A. Role of genetic background in somatic embryogenesis in Medicago. Plant Cell Tissue Organ Culture, 4:111-122, 1985.

[34] Parrot W.A. Auxin - stimulated somatic embryogenesis from immature cotyledons of white clover. Plant Cell Reports, 10: 17-21, 1991.

[35] Bencheikh M. & Galais A. Somatic embryogenesis in pea (*Pisum sativum* L and *Pisum arvense* L.): Diallel analysis and genetic control. Euphitica, 90 (3): 257-267, 1996.

[36] Bingham E.T., Hurley L.V., Kaatz D.M. & Saunders J.W. Breeding alfalfa which regenerates from callus tissue in culture. Crop Sciience, 12: 719-721, 1975.

[37] Reisch J.W. & Bingham E.T. The genetic control of bud formation from callus cultures of diploid alfalfa. Plant Science Letters, 20: 71-77, 1980.

[38] Sardinha R.M.A., Bessa A.M.S., Blake J., Guyyer D., Cassami C. & Tamba-Bungue P. Selection of superior genotypes of cashew (*Anacardium occidentale* L.) in Guinea-Bissau and the development of in vitro technique for their propagation. Final Summary Rept., Contract TS-2-A-01, Lisbon, Portugal, 67p, 1993.