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EVALUATION OF *IN VITRO* PROTOCOLS FOR EFFECTIVE REGENERATION OF WEST AFRICAN *Theobroma cocoa* (L)

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ABSTRACT. Cacao is a perennial tree crop from the Malvaceae family. It is made up of twenty-one species, with *Theobroma cacao* as the commonest and one of the most economically important crops globally. West Africa is the largest region of producers. Lack of availability of planting materials all year round has been a major factor besetting cocoa production in West Africa and has led to a drastic reduction in production in the last decade. Establishing an efficient *in vitro* protocol for germinating zygotic embryos of local West African cocoa varieties offers a solution to expedite large-scale planting material production to enhance cocoa sustainable production and material availability. An effective *in vitro* protocol for germinating zygotic embryos of local West African cocoa varieties will help produce large planting materials within the shortest possible time and promote sustainable supply, which could boost cocoa production within the region and increase the availability of planting materials. Four West African local varieties of *Theobroma cacao* were cultured in three explant types on both Driver and Kuniyaki Walnut (DKW) basal salts and Murashige and Skoog (MS) culture media without growth regulators for twenty days. The three explant types included seeds with mucilage (SWM), seeds without mucilage (SWtM) and embryo axis (EA). The cultured explants were kept in a growth room of light intensity with a temperature of 25 ℃ ±1, relative humidity of 85% and the three explant types responded differently.

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Germination responses significantly varied among the explant types. SWtM sprouted earlier with more than 160 plantlets before day 5, followed by EA with 150 plantlets before day 5, while SWM showed the lowest mean germination percentage of 8% compared to SWtM and EA with 9% Early sprouting was observed in the DKW medium with almost 250 (34.72%) plantlets development before day five compared to the MS medium with less than 150 (20.83%) plantlets development, but the MS medium produces the optimal growth performance with the best mean germination time of 0.08 per day, final germination percentage of 71.39%, and development of the growth parameters leading to the development of plantlets, including 2.60 for number of leaves. This study shows that the media for *in vitro* culture and the source of explants significantly influence seed germination and produce dissimilar effects on the germination of zygotic embryos of *Theobroma cacao* varieties. The study recommends using SWtM on DKW medium for early sprouting of seeds of *Theobroma cacao* followed by a transfer into MS medium for seedling development into plants.

Keywords: DKW; explants types; germination; *in vitro*; media types; MS; *Theobroma cacao*.

INTRODUCTION

West Africa is the largest producer of cocoa (*Theobroma cacao L*), a perennial plant that produces cocoa beans. Despite the challenges posed by the COVID-19 pandemic, global production for 2020/2021 reached an estimated 5.02 million tons, with Africa contributing nearly 70 % of this production (ICCO, 2021; Shahbandeh, 2021). Within West Africa, Cote d'Ivoire, Ghana, and Nigeria emerge as three of the top five global cocoa producers, accounting for 40%, 18%, and 6.8% of the 2020/2021 worldwide production projection, respectively (Shahbandeh, 2021). This crop supports over 6 million farmers and provides livelihoods for approximately 40 million individuals globally (World Cocoa Foundation, 2012).

Cocoa offers several beneficial properties for human nutrition, and health and an unforgettable history in many cultures with important economic and social implications globally. Studies by Tomaru *et al.* (2007); Pucciarelli and Grivetti (2008) and Selmi *et al.* (2008) highlighted its efficacy of treating upper respiratory tract problems, including colds and coughs as well as providing enhanced mental well-being and protection against nutritional deficiencies (which remains a major problem for many developing regions, such as West Africa). These properties among others placed the plant as a highly valued product (Araujo *et al.*, 2014).

The increasing international demand for cocoa has prompted countries within the region, including Liberia to explore production, while leading cocoa-producing nations are intensifying efforts to rehabilitate and boost production (Amoako *et al.,* 2019; Karmo, 2020). This means, the need for seedling production is rapidly increasing, which is largely proportional to huge quantities of topsoil needed for nursery work. Such demand for topsoil to meet this demand will eventually lead to serious degradation of productive farmland. Nowadays, climate change has further compounded this challenge by limiting suitable land for cocoa seedling development.

Despite its leading global production status, West Africa faces numerous obstacles such as susceptibility to pests and diseases, delayed maturation due to climate change, and insufficient use of inputs resulting from inadequate access to improved planting materials (Wessel and Quist-Wessel, 2015). Intensive and targeted breeding programs have been proposed as a viable solution to address these challenges and enhance cocoa production in the region (Aikpokpodion, 2011).

However, the region's heavy reliance on nurseries, predominantly composed of topsoil in shaded locations, exacerbates the scarcity of topsoil resources (Amoako *et al.,* 2019). Cocoa breeding, which typically spans 2-3 years, requires a significant time and cash investment. Advances in agriculture, particularly in crop improvement, genetic editing, and reduced breeding periods, offer promising solutions. Tissue culture, in particular, enables year-round production through micropropagation, providing a sustainable supply of planting materials (Olasupo and Aikpokpodion, 2019).

With the advancement in science, agriculture has witnessed substantial development, particularly in areas such as crop improvement, genetic base editing, resistance to pests and diseases, climate change, and reduction in breeding and production periods.

Moreover, the ability to achieve year-round production through micropropagation offers a sustainable solution for future supply. These advancements have been made possible through tissue culture, a technique involving the growth of entire plants from small pieces of plant tissue in a medium (Reece *et al.,* 2011).

Notably, all tissue culture techniques heavily rely on the development of efficient plant regeneration protocols, which is simply only possible through the availability of healthy explants. This can be derived from *in vitro* seedlings, fortunately, *in vitro* germination of seeds can easily produce young and sterile plant material that can be further propagated using *in vitro* propagation protocols (Koné *et al.,* 2010).

This means seedlings obtained *in vitro* can be directly used for other tissue culture experiments without any sterilization as well as an efficient tool for obtaining large numbers of individuals free of contaminating sources (Hung and Trueman, 1980; Silva *et al.*, 2010; 2011) since its main purpose is for further micropropagation and regeneration.

The transition from seed sterilization to *in vitro* proliferation is crucial for the development of sanitary *in vitro* plantlets, reducing contaminants and enhancing explant survival rates (Srivastava *et al.,* 2010).

This approach is beneficial for population establishment and conservation, addressing the high demand for planting materials, as conventional seed and vegetative cuttings propagation may prove insufficient or unreliable (Samuel *et al.*, 2009). Additionally, tissue culture serves as a promising tool for germplasm collections, international breeding initiatives, and distribution efforts, with the potential to augment all other propagation methods (Esan, 1977; Dias, 2001).

MATERIALS AND METHODS

Pods sources and surface sterilization

Four cocoa genotypes of cocoa pods, including CRIN TC-3 to TC-5 varieties and an unidentified variety, were obtained from the Cocoa Research Institute of Nigeria (CRIN) and the Bodija market in Ibadan, Nigeria. The pod surfaces were sterilized by washing with soap and tap water, followed by rinsing with distilled water. Subsequently, they were stored at room temperature under a laminar airflow cabinet, and the pods were sequentially surface-sterilized with 70% ethyl alcohol for 45 seconds.

Obtaining of seeds

Pods were broken open with a knife sterilized by autoclaving, and then bathed in 70% ethyl alcohol. Seeds were carefully removed from pods and prepared into three different explant types (*Figure 1*), seeds with mucilage (SWM) (Figure 1a), seeds without mucilage (SWtM) (*Figure 1b*) and embryo axis (EA) (*Figure 1c*), and subsequently cultured in the media (MS and DKW).

Media composition

The three explant types were cultured on Driver and Kuniyaki Walnut (DKW) basal salts and Murashige and Skoog (MS) media, with sugar (30 g/L), Myo-inositol (0.1 g/L) and no growth regulators.

The pH of each culture media was adjusted to 5.7 before adding before adding agar (7.518 g/L). Each media was autoclaved at 15 psi and 121 ℃ for 15 minutes.

Media strength

To optimize germination and the development of plantlets *in vitro*, the explant types were induced on full DKW and MS media, at concentrations of 5.32 g/L and 4.43 g/L, respectively.

Culture conditions

Cultured plants were maintained in a growth room with a temperature of 25 ℃ ±1 and relative humidity of 85%. The photoperiod consisted of 16 hours of light and 8 hours of darkness.

Light was provided by cool white fluorescent bulbs with a light intensity of $25\mu mol^{-1}m^{-1}$.

1a. SWM 1b. SWtM 1c. EA

Figure 1 – The three different explant types of *Theobroma cacao* seeds sprouting

Data collection on germination and growth parameters

Germination was considered to have taken place with the appearance of a 2 mm radicle and plumule as described by Koné, *et al.* (2015). Data was recorded for each seed treatment, from the day after sowing on each media type. The germination period was determined by the number of days from sowing to seedling emergence (Fetouh and Hassan, 2014).

The total germination period of twenty days and the mean germination time (MGT) were calculated using the formula cited by Ellis and Roberts (1981), given as MGT = ∑(nT)/∑n. Where "n" represents the number of seeds newly germinated at a time at 25 ℃; *T* represents the hours from the beginning of the germination test and ∑n is the final germination. The total number of germinated seeds for each explant as well as each media type was summed up to determine the cumulative germination separately (Koné, *et al.,* 2015). The rate of germination was calculated using the Czabator (1962) procedure. After twenty days of sowing, germinated plantlets were removed from the media, and the height of the seedling's, the number of roots and the number of leaves were recorded.

Data analysis and experimental design

A complete randomized design with ten seeds per treatment for three explant types was employed, and this was repeated thrice. The data were subjected to analysis of variance (ANOVA) to detect significant differences, and means were considered significantly different at $P \leq 0.05$ using the Duncan Multiple Range Test (DMRT) and R software, version 4.1.0 package (R Core Team, 2022).

RESULTS AND DISCUSSIONS

Observation of *Theobroma cacao* plantlets survival after 20 days of culture

After 20 days of culture, approximately 498 seeds, accounting for 69.2 % of the total seeds, survived on both culture media (*Table 1*). This study aligns with previous research highlighting the efficacy of tissue culture technology in enhancing *in vitro* seed germination and seedling establishment (Shahzad and Sahai, 2014). Furthermore, it corroborates with findings by Pence *et al.* (1979), who demonstrated that tissue explants of cocoa could be initiated on any tissue culture media without the need for growth regulators. The survival of plants was directly influenced by the type of explants used. Specifically, the number of surviving SWtM totalled 176 (35.34%), while explants with mucilage (SWM) and embryonic axis (EA) had 164 (32.93%), and 158 (31.73%) survivals, respectively. This suggests that SWtM had a more favorable impact on the *in vitro* germination of cacao seeds. These results support the notion that explant types are among the various factors that influence the multiplication and germination of plants *in vitro* (Das *et al.,* 1995).

Effect of different culture media on the survival of *Theobroma cacao* plantlets after 20 days

One of the most important biotic and abiotic factors influencing plant regeneration and germination is the

culture media (Shahzad, *et al.,* 2017; Murashige and Skoog, 1962). However, it was observed that the total number of plantlets that were developed on the MS media was 262 (52.61%) (*Table 1*). In contrast, DKW media induced the development of 236 (47.39%) plantlets. This demonstrates that MS has a greater influence on germinating cocoa seeds than DKW. This is consistent with Ajijah's (2016) findings, which suggest that tissue culture media composition (*Table 2*) determines *in vitro* regeneration success.

Effects of treatment on the early sprouting of explants of *Theobroma cacao* seeds

As shown in *Figure 2 (a)*, DKW media produced explants close to 250 (34.72%) before day five, while MS media produced explants little over 150 (20.83%) before day five and on day five, subsequently, MS media induced sprouting of more than 50 (6.94%) explants while DKW induced the sprouting of less than 50 (6.94%) explants. Both media induced less than 50 sprouting of explants after day five. This proves that the DKW media has a greater influence on early sprouting (before the 5th day) compared to MS media which influenced more germination from the fifth day onward. The ability of DKW to influence early sprouting agrees with the report of Li *et al.* (1998). In this report, callus was induced on the flower crown and staminodes using the primary callus induction medium of DKW for 14 days.

Additionally, an experiment that verified the success of different culture media (DKW, MS, WPM and NGE) conducted on *Juglans spp.*, showed that DKW media, though not the best performer, outshined MS with 54% to 27%, respectively within 10 to 15 days of the total 35 days of inducement period (Sánchez-Zamora *et al.*, 2006). According to Shiran *et al.* (2019), MS media of different concentration levels cannot influence early rooting in cacao. A 1/4 MS and 1/10 MS media would initiate shoot on the 10th day and 1/2 MS media would initiate shoot formation by the 15th day in a 22-day inoculation period.

Also, in a DKW medium supplemented with Kinetin and 2,4-D, callus formation occurred 5-7 days after culture and was preceded by swelling explants with all responsive explants forming callus after two weeks in culture (Ajijah *et al.*, 2016). The study further revealed that both supplements and their interaction with explant types did not significantly affect callus formation.

Table 1 – Observation of *Theobroma cacao* plantlet survival after 20 days of culturing

Explant Types	Media Types					
	No. Cultured		No. of Survival		No. of Non-survival	
	DKW	MS	DKW	MS	DKW	MS
SWM	120	120	80	84	40	36
SWtM	120	120	83	93	37	27
EA	120	120	73	85	47	35
Total	360	360	236	262	124	98
Mean %			65.56	72.78	34.44	27.22

No.: Number; SWM: Seeds with Mucilage; SWtM: Seeds without Mucilage;
EA: Embryo Axis; MS: Murashige and Skoog; DKW: Driver and Kuniyaki Walnut.

Table 2 – Composition of MS and DKW media types used

Nutrients components	Concentration of Media Types (mg/L)	
	MS (4430 mg)	DKW (5320 mg)
Inorganic source		
Ammonia nitrate	1650.0	1416.0
Potassium nitrate	1900.0	-
Calcium nitrate	-	1367.0
Magnesium sulfate heptahydrate	370.0	361.49
Potassium sulfate	-	1559.0
Nickel Sulfate hexahydrate	-	0.005
Calcium chloride dehydrate	440.0	112.5
Potassium phosphate	170.0	265.0
Manganese sulfate monohydrate	-	33.5
Zinc sulfate heptahydrate	8.6	-
Zinc nitrate hexahydrate		17.0
Boric acid	6.2	4.8
Potassium iodide	0.83	
Sodium molybdate dehydrate	0.25	0.39
Cupric sulfate pentahydrate	0.25	0.25
Iron source		
Ferric sulfate heptahydrate	27.8	33.8
Sodium ethylene dinitrotetraacetic acid	37.3	45.4
Vitamins source		
Myo-inositol	100.0	100.0
Glycine	2.0	2.0
Pyridoxine hydrochloride	0.5	0.5
Nicotine acid	0.5	0.5
Thiamine	0.1	0.1
Organic solids		
Sugars	30000	30000
Agars	7000	7000

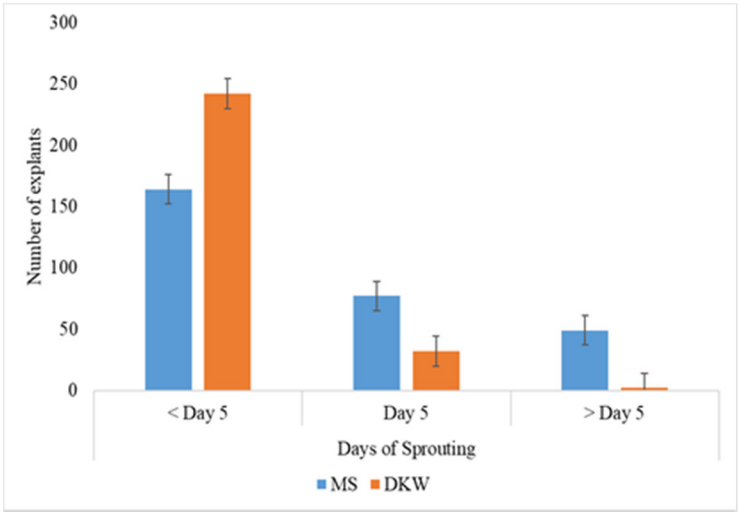
DKW: Driver and Kuniyaki Walnut; MS: Murashige and Skoog; Mg: Milligram and L: Liters

In *Figure 1 (b)*, seeds treated as SWtM sprouted a little over 160 before day 5, while seeds treated as EA sprouted below 150 before day 5 and those treated as SWM sprouted with a total of 100 before day 5. Moreover, on day five, the seeds treated as SWM sprouted with a total of 60, while seeds treated as both SWtM and EA sprouted a little over 20. Additionally, after day five, all the seeds treated as SWM sprouted a little above 20, while SWtM and EA explants treatments sprouted below 20 with EA spouting more than SWtM. These results showed that SWtM germinates early compared to the other two explant types, with EA considered to be the next in line, and SWM exhibiting the slowest germination among all three.

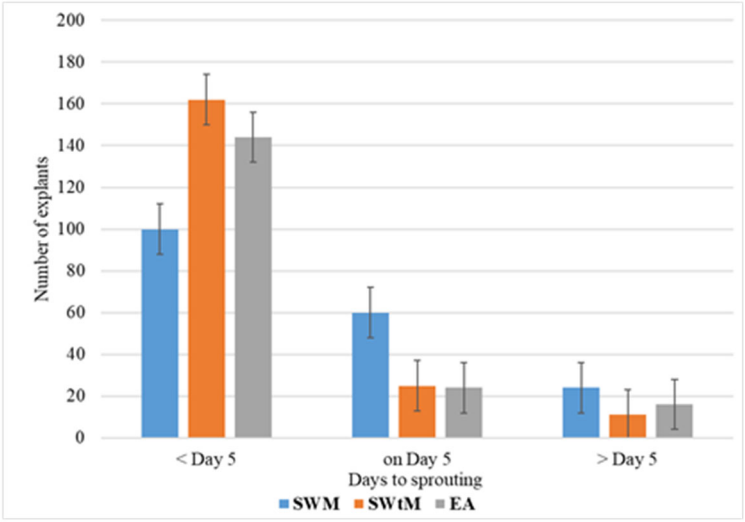
Many studies have reported findings that EA sprouts earlier compared to SWtM. For instance, Shiran *et al.,* (2019) observed that embryonic axes emerge radicle within five days in germinating cocoa seeds, regardless of the size of cotyledons. Additionally, studies by,

Kone *et al.* (2015), Adu-Dapaah and Sangwan, (2004), Ochatt *et al.,* (2002) and Maliro and Kwapata, (2000) have consistently reported that EA exhibits the shortest germination time of 4-5 days compared to other seed explants.

This study suggests otherwise and reports that SWtM exhibited the shortest germination time compared to EA. The delay in germination observed in EA compared to SWtM could be attributed to the presence of hard outer seed coat layers, which often delay seed germination due to impermeability and restriction of radical emergence, a phenomenon also applicable to SWM.



(a) Effect of Media types



(b) Effect of Explant types

Figure 2 – Response to early sprouting

Additionally, the delay in EA germination compared to SWtM may be due to the maturity level of the embryos isolated from seeds. This observation is consistent with the findings by Samuel *et al.* (2009) and Fregene *et al.* (1999) in studies on embryo cultures of *Givotia rottleriformis Griff* and cassava, respectively. It is important to note that this study did not assess the physiological growth stage of the embryos before experimentation.

Treatment effects on the development of growth parameters of *Theobroma cacao* plantlets

The combinations of the four genotypes and three different explant types had a significant difference in the number of leaves developed (*Table 3*). This finding aligns with Jacoba du Plessis *et al.,* (2020), who investigated *in vitro* seed germination and seedling performance of *Hibiscus coddii subsp.* Barnardii showed no significant differences among the three different cultures for any of the germination parameters. However, this result contrasts with the findings of Shahzad and Sahai (2014), who reported that regenerative or growth responses were genotype-dependent for *in vitro* seed germination and establishment of *Balanites aegyptiaca* (L.).

A study on growth inhibition as a viable technique to enhance the storage of synthetic seeds of *Theobroma cocoa* stored in different media demonstrated significant differences in radicle emergence and shoot regeneration (Shiran *et al.,* 2019). However, like many other studies, including the current work; there has not been a conclusive and optimized observation of the effects of the genotypes and explants or media types for *in vitro* germination.

Treatment effects on leaves development of *Theobroma cacao* plantlets

There was no significant difference among the four *Theobroma cacao* genotypes in terms of mean separation regarding the development of the numbers of leaves (*Table 4*). *Theobroma cacao* genotype (CRIN TC-3) had more leaves (2.79) than CRIN TC-4 and unknown varieties had 2.46 each. CRIN TC-5 had the lowest number of leaves (2.32).

While there have not been many reports on genotype influences on *in vitro* germination of seeds, including *T. cacao* works on other tissue culture techniques such as somatic embryogenesis, have indicated significant genotype effects.

Regardless of genotypes, all three explant types exhibited a significant difference in the number of roots of *Theobroma cacao* plantlets. The impermeability of the seed coat to water and oxygen, which are primary factors for successful germination, contributes to this variation (Baskin and Baskin, 1988). However, the explant treated as SWtM displayed more leaves during germination (2.77) compared to SWM, but fewer leaves during germination (2.53) compared to EA (2.18). These results align with the findings of Koné *et al.* (2015) on seedling growth of Bambara groundnut seeds.

Regardless of the medium, explants with and without seed coats produced high numbers of leaves, plantlet height, root length and biomass.

Table 3 – Treatment effect on radicle and seedling height, number of leaves and roots of *Theobroma cacao* seeds germination

Source of variance	df.	Mean sq.			
		radicle_leng.	Seedling_ht.	no. of leaves	no. of roots
Rep	2	48.74^{NS}	71.770^{NS}	0.4056^{NS}	359.8^{NS}
Gen	3	12.16^{NS}	9.812^{NS}	7.7444^{NS}	276.2^{NS}
Exp_t.	2	97.10^{NS}	185.251^{NS}	21.2764^{NS}	4284.3^{NS}
Media_t.	1	26.32^{NS}	75.184^{NS}	8.4500^{NS}	177.0^{NS}
Gen x Exp_t.	6	1.97^{NS}	1.738^{NS}	1.6264^{S}	48.4^{NS}
Gen x Media_t.	3	0.81^{NS}	1.390^{NS}	0.2981^{NS}	33.8^{NS}
Exp_t. x Media_t	2	4.32^{NS}	7.675^{NS}	5.1542^{NS}	173.3^{NS}
Gen x Exp_t x Media_t	6	1.43^{NS}	3.414^{NS}	1.4968^{NS}	56.2^{NS}
Residuals	478	2.16	3.97	0.7194	70.8

Rep: Replications; Gen: Genotypes; Exp_t: Explant types; Media_t: Media types; df: degree of freedom; sq: square; Radicle_leng: Radicle length; Seedling_ht: Seedling height; No.: number; S: Significant at 5% probability and NS: Not significant 5% probability

Table 4 – The effects of treatment on the number of leaves of *Theobroma cacao* plantlets in tissue culture

Treatments	No. of Leaves
Genotypes	
CRIN *Theobroma cacao* -3	2.79^{a}
CRIN *Theobroma cacao* -4	2.46^{a}
Unknown varieties	2.46^{a}
CRIN *Theobroma cacao* -5	2.32^{a}
Explant types	
Seeds without Mucilage	2.77^{a}
Seeds with Mucilage	2.53^{b}
Embryo Axis	2.18^{c}
Media Types	
Murashige and Skoog	2.60^{a}
Driver and Kuniyaki Walnut	2.39^{b}

Means with superscripts of the same alphabet in the same column are not significantly different from each other, CRIN: Cocoa Research Institute of Nigeria and No.: Number.

Sheran *et al.* (2019), reported that regardless of EA size, it will delay shoots of cacao seeds, leading to delayed s seedling length and leaf formation. Therefore, the delay of shoot emergence could influence the development of the number of leaves. The superior performance of SWM compared to EA may be attributed to the presence of mucilage, which retains sufficient water, as continuous water supply is essential for initiating and completing the development process (Katembe *et al.,* 1998). There was a significant difference between the two media types. The Murashige and Skoog (MS) medium induced the formation of more leaves during *Theobroma cacao* germination compared to the Driver and Kuniyaki Walnut (DKW) basal medium. The means number of leaves for media types were 2.60 and 2.39, respectively. This

finding contrasts with Rahman (2018), who reported that DKW outperformed MS medium in terms of multiple shoot regeneration and callus-mediated shooting of *C. ternatea.* Ajijah *et al.,* (2016), reported that DKW medium enriched with kinetin resulted in significant somatic embryo development for *Theobroma cacao* regeneration, with 50% germination, and 65% plantlet conversion. Similarly, the regeneration of the Nigerian cassava (*Manihot esculenta* Crantz) varieties showed varying degrees of success, ranging from 10 to 80% for germination and 49% to 78% for plantlet conversion (Nkaa *et al.,* 2015).

DKW nutrient adjustment also enhanced hazelnut development in micro-propagated *Corylus avellana L.* (Hand and Reed, 2014). Conversely, MS medium was reported to be efficient in generating plant parts such as the number of leaves, root length, and biomass in various explant types, including embryo axis and seeds with coat, regardless of concentration or supplements (Kone *et al.,* 2015). Additionally, the current study showed across all explant types, except EA performed better in developing several leaves (*Figure 3*).

Effect of genotypes on mean germination time and final germination percentage of *Theobroma cacao* seeds

The mean germination time (MGT)regardless of explant treatment types, was significantly influenced by the four different genotypes (*Table 5*). Among the genotypes, CRIN TC-3 had the quickest mean germination period (11.38 days). Following TC-4 with the longest mean germination time of 11.81, CRIN TC-5 and UNKNV had mean germination times of 11.40 days and 11.64 days, respectively. Additionally, significant differences were observed in the final germination percentage (FGP), with CRIN TC-4 demonstrating the highest FGP (71.67%). The FGP for UKNV, CRIN TC-5, and CRIN TC-3 were 70.56%, 67.22%, and 63.33%, respectively. These results suggest that genotype performances are attributed to the genetic characteristics of different populations (Bewley and Black, 1994). According to Shahzad and Sahai (2014), MGT varies depending on the genotype, which is consistent with the findings of this study.

Effects of explant types treatment on the mean germination time and final germination percentage of *Theobroma cacao* seeds

The SWM had the lowest MGT of 0.08 and the second-highest FGP of 66.25%. Both SWtM and EA explants had 0.09 MGT. The SWtM explant had a greater FGP of 74.5% compared to the EA explant (65%). The MGT for the different types of explant was similar (Kone et al, 2015). Although EA and SWtM performed better than seed with a coat. This study also contradicts the findings of Shiran *et al.* (2019), who indicated that EA of all sizes will yield an early MGT.

Effects of media types treatment on the mean germination time and final germination percentage of *Theobroma cacao* seeds

The MS medium had a significant effect on FGP (71.39%) compared to 65.83% for the DKW medium. The effects of both media treatments on the MGT are significantly different.

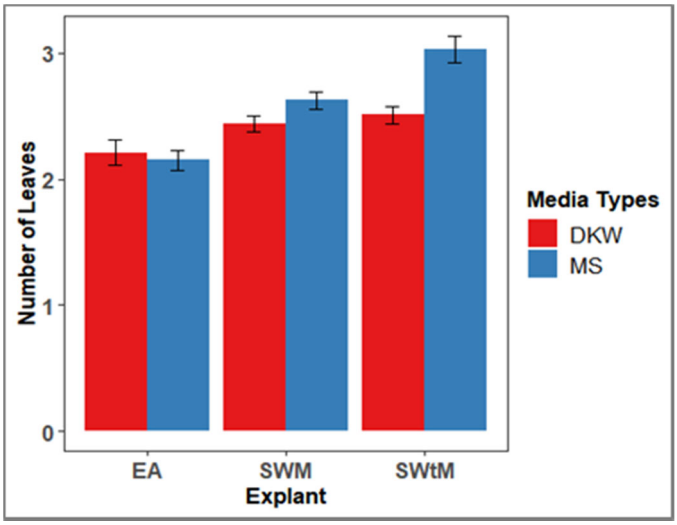


Figure 3 – Effects of explant types and media treatment on the number of leaves of *Theobroma cacao* plantlets developed *in vitro* tissue culture

Table 5 – Effects of treatments on mean germination time of *Theobroma cacao* seeds *in vitro*

Treatments	MGT for (20 DAYS)	FGP (%)
Genotypes		
CRIN *Theobroma cacao* -3	11.38[a]	63.33[c]
CRIN *Theobroma cacao* -5	11.40[a]	67.22[b]
Unknown varieties	11.64[a]	70.56[a]
CRIN *Theobroma cacao* -4	11.81[a]	71.67[a]
Explant Types		
Seeds with Mucilage	0.08[a]	66.25[b]
Seeds without Mucilage	0.09[a]	74.58[a]
Embryo Axis	0.09[a]	65.00[ab]
Media Types		
Murashige and Skoog	0.08[a]	71.39[a]
Driver and Kuniyaki Walnut	0.09[a]	65.83[b]

MGT: Mean germination time; FGP: Final germination time, CRIN: Cocoa Research Institute of Nigeria and Means with superscripts of the same alphabet in the same column are not significantly different from each other.

This study contradicts the findings of Kone *et al.* (2015), who showed that the various culture media did not affect MGT. It should be emphasized that the additional 3% sucrose supplements employed by Kone *et al.* (2015) as well as their disdain for DKW, could explain the resulting outcome.

Nonetheless, the results agree with Kone *et al.,* (2015) on FGP.

CONCLUSIONS AND RECOMMENDATION

The study on *in vitro* germination of cocoa seeds shows a clear potential for efficiently producing plantlets within a short period, which could significantly contribute to meeting the increasing demand for planting materials by farmers. The crop, once hugely and

rapidly produced could serve as a viable alternative toward reawakening many nations, including nations of West Africa, since the production is driven by smallholder farmers. The composition of media composition for germination reveals that neither DKW nor MS media significantly influences the germination capacity of the three explant types tested. However, MS media exhibits better mean performance in promoting the development of the number of leaves, a crucial organ for photosynthesis.

Conversely, medium facilitates early sprouting, while MS medium demonstrates better performance in terms of mean germination time. Additionally, seeds without mucilage show faster protrusion, further highlighting the nuanced effects of media types on germination dynamics. These findings underscore the importance of optimizing media composition and explant types to enhance the efficiency of cocoa seed germination *in vitro*. By understanding the specific effects of different media types on germination parameters, researchers and agricultural practitioners can tailor cultivation practices to maximize plantlet production and ultimately contribute to a sustainable cocoa production system

From the observations, the authors recommend that research institutes, scientists, and government institutes should prioritize the research of similar studies and optimize discovered protocols.

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