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Effect of different concentrations of plant growth hormones on callus induction and regeneration of Shea tree (*Vitellaria paradoxa*)

Aguwa I.[1]*, Gana A. S.[1], Salaudeen M. T.[1], Tolorunse K. D.[1], Nmadu J. N.[2], Baba K. M.[2], Osunde Z. D.[3] and Okafor J. O.[4]

[1]Department of Crop Production, Federal University of Technology, Minna, Nigeria.
[2]Department of Agricultural Economics and Farm Management, Federal University of Technology, Minna, Nigeria.
[3]Department of Agriculture and Bioresources Engineering, Federal University of Technology, Minna, Nigeria.
[4]Department of Chemical Engineering, Federal University of Technology, Minna, Nigeria.

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The slow natural regeneration pattern of the Shea tree due to the long gestation period and intense harvest has limited the domestication and genetic improvement of this tree. These limitations have necessitated the need for an alternative method of conserving the Shea tree outside the natural habitat. The propagation of the Shea tree by the *in-vitro* clonal technique presents such an alternative method. The purpose of this study was to determine the optimal concentration of 2, 4-dichlorophenoxyacetic acid (2, 4-D) and Picloram in Murashige and Skoog medium for callus formation and regeneration. The first experiment was done to achieve the best surface sterilization method and the effect of different concentrations of 2, 4-D or Picloram on callus formation. Callus induction percentage (CI%) of the explants in Murashige and Skoog medium were evaluated. The basal media were supplemented with 30 g/L of sucrose, 2.8 g/L phytagel and combinations of 2, 4-D or Picloram in various concentrations (1.5, 2.0, 2.5, 3.0, 3.5, 4.0, and 4.5 mg/L) replicated four times with five explants in each bottle. From the result, the leaf explants soaked in 70% ethanol for 1 min and 1% sodium hypochlorite for 15 min with 1 µl of tween 20 had the highest percentage (100%) of sterile leaf explants and showed no contaminations both in the leaf and media. Callus was induced at 2 weeks of culturing in all the treatments except for MS basal without growth hormone which induced no callus. A concentration of 1.5 mg/ L 2, 4-D gave the best callus. The highest CI% (100%) was shown at 4 weeks in MS + 3.5 mg/L picloram media. The callus was light in colour and friable in texture. The result indicated that Picloram gave better callus compared to other treatments and will give a better response for regeneration of Shea tree.

Key words: Explant, callus induction, 2, 4-d, picloram, shea tree and Regeneration.

INTRODUCTION

Shea tree (*Vitellaria paradoxa*) belongs to the family Sapotaceae. It grows widely in the savannah region of West African Countries (Maranz and Wiesman, 2003). It is a deciduous dicotyledonous crop that has a gestation period varying from 15 to 20 years. The Shea tree is of great economic importance due to its multipurpose use (Yakubu et al., 2015). It serves as a fruit tree as well as an oilseed crop. It is popular for the Shea butter produced from it, which is relevant in the food, cosmetic and pharmaceutical industries (Boffa, 2015). Plant hormones are chemicals that regulate plant growth. They are signal molecules produced at a specific location in the plant and

extremely low concentrations. Hormones are naturally produced within plants, though very similar chemicals are produced by fungi and bacteria that can affect plant growth (Srivastava, 2002). The most widely used plant growth hormones are auxins and cytokinins. The types, concentrations and the ratio of these growth regulators used for media supplementation are essential for the type of culture responses obtained (Anca, 2009). Naturally, the Shea tree grows and regenerates itself in the wild but its slow and poor natural regeneration pattern due to long gestation period; impacts of bush fires and desertification have limited the domestication and genetic improvement of this crop. These limitations have necessitated the need for an alternative method of conserving this plant's genetic resource outside the natural habitat. The propagation of the Shea tree by the *in-vitro* clonal technique presents such an alternative method (Lovett and Haq, 2013). However, vegetative methods, such as grafting, budding, cuttings and air-layering, have only produced limited success (Yeboah et al., 2010). The micropropagation of woody tree species is now a method widely used in the regeneration and conservation of germplasms (Lovett and Haq, 2013). Callus induction and plant regeneration are some of the key tools in plant biotechnology that exploits the totipotent nature of plant cells (Mukherjee et al., 2011).

Tissue culture serves as an indispensable tool for transgenic plant production. For nearly any transformation system, an efficient regeneration protocol is imperative (Cardoza, 2008). Due to the lack of proper cultivation practices, destruction of plant habitats, excessive and indiscriminate collection of medicinal plants for the supplement of global demands on herbal medicine, many medicinal plants like *Salacia chinensis* and *Vitelleria paradoxa* are severely threatened. Therefore, to conserve and rapidly propagate the rare and endangered medicinal plants, advanced biotechnological methods of culturing plant cells and tissues like micro propagation methods are employed (Nalawade et al., 2001). This study aims to evaluate the effect of different concentrations of plant growth hormones on callus induction and regeneration of Shea trees. Also, to produce callus from explant of the Shea tree and determine the optimal concentration of 2, 4-D and Picloram media for callus formation and plantlet regeneration.

MATERIALS AND METHODS

Study location

The study was carried out in Agricultural Biotechnology Laboratory at Science and Technology Complex (SHETSCO), Sheda, located at the outskirt of Abuja, North Central Nigeria with Latitude 8˚51'25' N and Longitude 7˚02'39''E.

Plant material used for callus induction

A three days old tender leaf of Shea butter was used as an explant for the callus induction.

Treatment and experimental design

The treatments consist of six different concentrations of 2, 4 –D and Picloram (4.5, 4, 3.5, 3, 2.5, and 1.5 mg/L) arranged in a completely randomised design (CRD) with four replications.

Explant preparation and surface sterilization.

The three days old leave explants were plucked off from the mother plant using a sterile knife and then taken to the laboratory. The explants were washed vigorously under running tap water with drops of liquid detergent to remove the dirt particles then followed by rinsing with distilled water. The laminar flow hood was sprinkled with 70% Ethanol to control contamination and the burner was lit. To surface sterilize the tender leaves used as explants, ethanol and sodium hypochlorite method of surface sterilization were adopted. However, due to lack of standing protocol for surface sterilization of Shea butter explants for callus induction, three different ethanol and sodium hypochlorite combinations were used with varying concentrations and timing as follows:

S0: Explants were only washed with distilled water 5-6 times.
S1: Explants soaked in 70% ethanol for 1 min then soaked in 1% sodium hypochlorite for 15 min with 1 µl of tween 20.
S2: Explants were soaked in 70% ethanol for 2 min then soaked in 2% sodium hypochlorite for 20 min with 1µl of tween 20.
S3: Explants were soaked in 70% ethanol for 3 min then soaked in 3% sodium hypochlorite for 10 min with 1µl of tween 20.

Using the surface sterilization methods above, the explants were soaked in 70% ethanol first at S1, S2 and S3, then rinsed three times with sterile water. Secondly, the explants were soaked in sodium hypochlorite with 1 µl tween 20 then rinsed four times with sterile water. After surface sterilization, all explants were trimmed to small sizes of 2 mm by 2 mm and then cultured on MS medium without growth hormone to check out for contaminations and sterility within four days. Percentage sterility was recorded.

Preparation of MS media and callus induction

Two different plant growth regulators (PGRs; 2, 4-D and Picloram) was used for callus induction in shea butter. Six different concentrations of each auxin (4.5, 4, 3.5, 3, 2.5, and 1.5 mg/L) were used in Murashige and Skoog media (Murashige and Skoog 1962) as a basal medium and a control treatment with no growth hormone was included (Figure 2). Murashige and Skoog culture medium (MS) was supplemented with 30 g/L sucrose, as a support material (Khan et al., 2001) and 100 g/l ascorbic acid. The pH was adjusted

*Corresponding author. E-mail: ifeomaaguwa@gmail.com. Tel. +234 8062831696.

to 5.7 using 1 M of NaOH. Pyhtagel was added as gelling age the medium at a concentration of 2.8 g/L before the medium was sterilized at psi 121°C for 15 min. At the end of the sterilization, the media was allowed to cool. Different concentrations of the auxins (2, 4-D or Picloram) was added using a microfilter and the medium was dispensed into the bottle in the laminar flow hood and allowed to settle for 24 h. Sterile explants were cultured into the bottle and placed about 2 cm apart on the culture medium. Twenty explants were used in all the media with each bottle having five explants and four bottles per media. The bottle was sealed in a polythene bag and stored in the dark growth room 28°C. The two sets of the experiment (MS + 2, 4-D and MS + Picloram) was conducted concurrently. The performance of the culture and callus produced was evaluated visually at 2 and 4 weeks and recorded. The cultures were monitored, every four weeks for sub-culturing and development noted. After four weeks, the explants were separated from the callus formed and the callus was moved into a fresh media for regeneration. The criteria for scoring explants for callus is based on the number of explants producing callus per media according to Amoo and Ayisire (2005).

Data collections

1. Percentage sterility was calculated using the formula:

$$\text{Percentage sterility} = \frac{\text{Number of Uncontaminated explants}}{\text{Total number of cultured explants}} \times 100$$

(Nwala, 2012).

2. Callus Induction Percentage: Percentage of callus induction was calculated using the formula:

$$\text{Callus Induction Percentage (\%)} = \frac{\text{Number of explants with callus}}{\text{Total number of explants inoculated}} \times 100$$ (Kabir et al., 2008).

3. Degree of callus formed was rated using the scale; **+** = Very Poor, **+ +** = poor, **+ + +** = Good, **+ + + +** = Very good (Dhiya et al., 2013).

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) using statistical analysis software (SAS). Means were separated using Duncan's multiple range test (DMRT) at a 5% level of probability.

RESULTS

Effect of different sterilant combinations on leaf explants of *Vitelleria paradoxa* for tissue culture establishment

Table 1 summarizes the efficiency of the different combinations of ethanol and sodium hypochlorite at different times and concentrations. From the result, it was observed that the method of soaking the leaf explant in 70% ethanol for 3 min and then 3% sodium hypochlorite for 10 min had the lowest percentage (15%) of sterile leaves. Out of the 20 explants, only 3 explants were sterile while others were contaminated and burnt. The soaking of explants in a combination of 70% ethanol for 2 min and 2% of sodium hypochlorite for 20 min showed a higher percentage of 75% of sterile leaves. Leaf explants soaked in a combination of 70% ethanol for 1 min and 1% sodium hypochlorite for 15 min had the highest percentage (100%) of sterile leaf explants and shows no contaminations both in the leaf and media. The leaves remained green and sterile for ten days. Also, washing the leaf explants with only distilled water had no sterile leaf explants (Figure 1).

Effect of MS media supplemented with different concentrations of 2, 4 –D for callus formation on leaf explants at 2 weeks and 4 weeks interval.

From the observations, at 2 weeks all the media had at least one explant induced callus except for the MS basal (control) which had no growth regulator. At 2 and 4 weeks, media MA1 and MB1 had the least callus induction percentage of 5% and 10%, respectively. At 2 weeks, MD1 and ME1 had 20% same as the media MC1 and MD1 at 4 weeks; although, media ME1 performed better with percentage value of 30% at 4 weeks. However, MF1 performed better with (55%) than other treatments at 2 weeks while at 4 weeks, MF1 had the highest percentage value of 75% of callus formed. Tables 2 and 3 show the analysis of variance using 2, 4 –D on leaf explant of the Shea tree. There were significant differences among treatments at 2 weeks and 4 weeks of culturing. However, there was no significant (p> 0.05) difference at 2 weeks and 4 weeks in media MA1, MB1, MC1 and MD1. Media MA1 and MB1 had the lowest mean value of 0.25 at 2 weeks and 0.5 at 4 weeks. At 2 weeks. The differences between media MD1 and ME1 was not significant (p>0.05). Also, media MF1 had the highest mean value of 2.75 and 3.75 and was significantly (p> 0.05) different from others in 2 and 4 weeks. Media MA1 and MB1 at 2 and 4 weeks produced very poor calli while MC1, MD1 and ME1 produced poor calli. However, media MF1 had good calli at 2 weeks and then produce very good calli at 4 weeks. Only MS basal produced no callus at 2 weeks and 4 weeks. The colour and morphology of calli produced in media MA1, MB1, MC1, MD1 and ME1 were compact and whitish except for media MF1 which calli were friable and whitish.

Effect of MS media supplemented with different concentrations of picloram on callus formation from leaf explants of Shea tree at 2 and 4 weeks intervals

Tables 4 and 5, show a positive response of explants to the different concentrations of picloram at 2 weeks of culturing. Media MA2, ME2 and MF2 at 2 weeks had the least callus percentage induction value of 20% while at

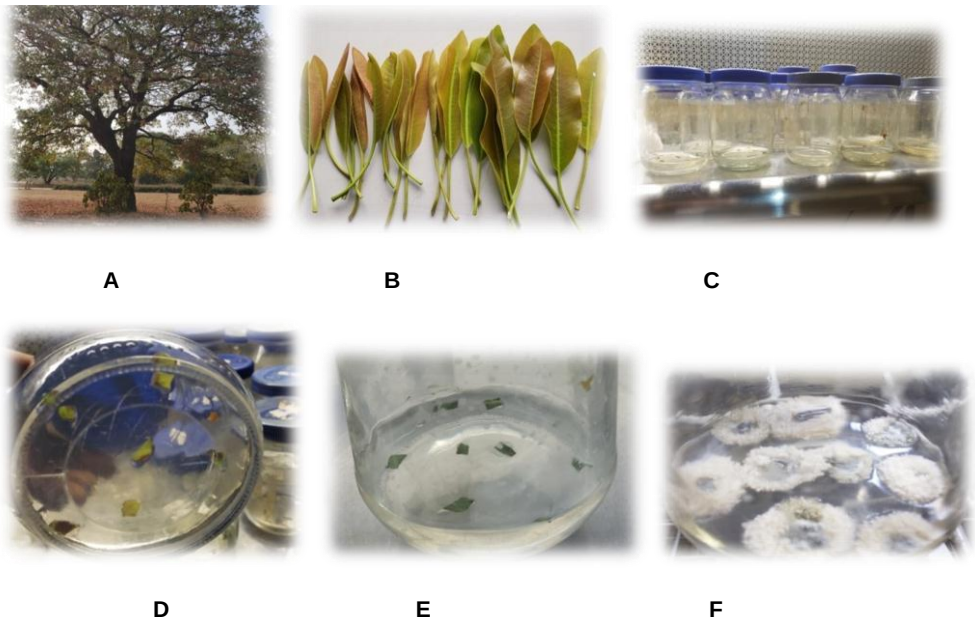

Figure 1. A- Source of explant (Mother plant), **B**- Leaf explants, **C-** Surface sterilized explants, **D-** Sterile explants, **E**- Sterile leaf explant, **F**- Contaminated explant.
Source: Authors

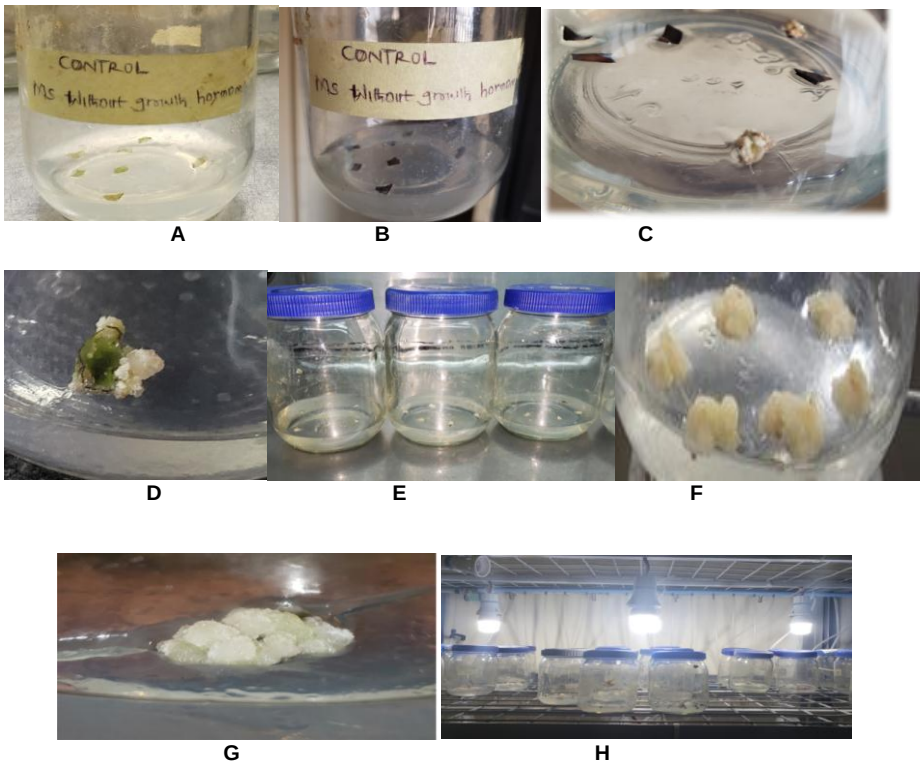

Figure 2. A- Explant in MS basal after culture, **B**- Explant in MS basal after 4 weeks, **C**- Callus from 4.5 mg/ L 2, 4 D, **D**- Callus from 3.5 mg/L Picloram, **E**- Callus subcultured for Multiplications, **F, G**- Callus multiplied in 3.0 mg/L Picloram after 4 weeks; **H**- The experiment in the growth culture room under light
Source: Authors

Table 1. Effects of different sterilant combinations on leaf explants of *Vitelleria paradoxa* for callus induction establishment.

Sterilant concentration/Time (mins)	No of healthy sterile leaves	% sterility of explants
S0 - 20 leaves soaked in distilled water and rinse 4 to 6 times	0	0
S1 - 20 leaves soaked in 70% ethanol for 1 min + 1% sodium hypochlorite for 15 min + 1 µl of tween 20	20	100
S2 - 20 leaves soaked in 70% ethanol for 2 min + 2% sodium hypochlorite for 20 min + 1 µl of tween 20	15	75
S3 - 20 leaves soaked in 70% ethanol for 3 min + 3% sodium hypochlorite for 10 min + 1 µl of tween 20	3	15

Source: Authors

Table 2. Effect of MS media supplemented with different concentrations of 2, 4-D on callus formation from leaf explants of Shea tree at 2 weeks.

Growth regulators 2,4-D	Callus induction percentage (%)	(Mean ± SE)	Degree of callus	Colour and morphology
Control	0	0	-	NC
MA1	5	0.25^{bc} ± 0.25	+	CW
MB1	5	0.25^{bc} ± 0.25	+	CW
MC1	15	0.75^{bc} ±0.25	+	CW
MD1	20	1.00^{b}±0.41	+ +	CW
ME1	20	1.00^{b}±0.41	+ +	CW
MF1	55	2.75^{a}±0.25	+ + +	FW

Mean with the same letters are not significantly different according to Duncan's multiple range test (DMRT) at p= 0.05. MA1 - MS + 4.5mg/L 2, 4-D, MB1- MS+ 4.0mg/L 2, 4-D, MC1- MS+ 3.5mg/L 2, 4-D, MD1- MS+ 3.0mg/L 2, 4-D, ME1- MS + 2.5mg/L 2, 4-D, MF1- MS+ 1.5mg/L 2, 4-D, FW- Friable White, CW- Compact White, - No Callus, + Very poor callus, ++ Poor callus, +++ Good callus, ++++ Very good callus.
Source: Authors

4 weeks media MA2, ME2 and MF2 had percentage value of 35%. Likewise, MB2 at 2 weeks but at 4 weeks, there was an increase in callus formed in ME2. However, at 2 weeks and 4 weeks MD2 performed better than all other treatments but media MC2 had the best result at 2 weeks and 4 weeks. At 4 weeks, media MC2 had the highest percentage value of 100% which gave the highest calli formed. In the mean value, there were significant (p<0.05) differences among the treatment. However, there were no differences at 2 weeks and 4 weeks in the media MA2, ME2 and MF2 respectively. At 2 weeks, media MA2, ME2 and MF2 gave the least mean value of 1.0 while at 4 weeks, media MA2, MF2 had the least mean value of 1.75; likewise media MB2 at 2 weeks. Also, at 2 weeks MC2 had the highest mean value of 3.75 and was significantly (p<0.05) different from others while at 4 weeks MC2 gave the highest mean value of 5.0 which is the best and significantly (p<0.05) different from others. From the observations, the degree of callus produced at 2 weeks from media MA2, MB2, ME2 and MF2 was poor likewise media MA2 and MF2 at 4 weeks. At 2 weeks, media MD2 produced good calli likewise media MB2 and MC2 at 4 weeks. Therefore, media MC2 and MD2 produced the best calli and was used for the regeneration of Shea plantlet. All the treatments MA2, MB2, MC2, MD2, ME2 and MF2 produced friable and whitish calli except for control which had no callus.

DISCUSSION

The first experiment was to investigate the suitable surface sterilization method for leaf explants of the Shea tree. It was observed that soaking the leaf explants in 70% ethanol for 1 min and 1% sodium hypochlorite for 15 mins with a drop of tween 20 gave the highest percentage (100%) of sterile leaves. This was also observed by (Ghosh, 2005), In the case of *Salacia chinensis* the surface sterilization with 70% ethanol for 1 min followed by sodium hypochlorite (1% + 1µl of Tween 20) for 15 min proved most effective for maximum survival percentage in leaf explants. Soaking the explants in 70% ethanol for 3 mins and 3% sodium hypochlorite for 10 mins had the lowest percentage (15%) of sterile leaf establishment because of the increased time in ethanol and increased concentration of sodium hypochlorite. It was also reported by Sharma et al. (2014) that increasing the time of exposure to sodium hypochlorite significantly reduced contamination but showed an adverse effect on explants. Also, when the

Table 3. Effect of MS media supplemented with different concentrations of 2, 4-D on callus formation from leaf explants of Shea tree at 4 weeks.

Growth regulators 2,4-D	Callus induction percentage (%)	(Mean ± SE)	Degree of callus	Colour and morphology
Control	0	0	-	NC
MA1	10	0.50^{cd}±0.29	+	CW
MB1	10	0.50^{cd} ±0.29	+	CW
MC1	20	1.00^{bc}±0.41	+ +	CW
MD1	20	1.00^{bc}±0.41	+ +	CW
ME1	30	1.50^{b}±0.29	+ +	CW
MF1	75	3.75^{a}±0.25	+ + + +	FW

Mean with same letters are not significantly different according to DMRT at p =0.05.
MA1- MS + 4.5mg/L 2, 4-D, MB1- MS+ 4.0mg/L 2, 4-D, MC1- MS+ 3.5mg/L 2, 4-D, MD1- MS+ 3.0mg/L 2, 4-D, ME1- MS + 2.5mg/L 2, 4-D, MF1- MS+ 1.5mg/L 2, 4-D, FW- Friable White CW- Compact White, - No Callus, + Very poor callus, ++ Poor callus, +++ Good callus, ++++ Very good callus.
Source: Authors

Table 4. Effect of MS media supplemented with different concentrations of picloram on callus formation from leaf explants of Shea tree at 2 weeks.

Growth regulators picloram	Callus induction percentage (%)	(Mean ± SE)	Degree of callus	Colour and morphology
Control	0	0	-	NC
MA2	20	1.00^{d}±0.0	+ +	FW
MB2	35	1.75^{c} ± 0.25	+ +	FW
MC2	75	3.75^{a}±0.25	+ + + +	FW
MD2	45	2.25^{b}±0.25	+ + +	FW
ME2	20	1.00^{d}±0.0	+ +	FW
MF2	20	1.00^{d}±0.0	+ +	FW

Mean with the same letters are not significantly different according to DMRT at *p* =0.05.
MA1- MS + 4.5mg/L Picloram, MB1- MS+ 4.0mg/L Picloram, MC1- MS+ 3.5mg/L Picloram, MD1- MS+ 3.0mg/L Picloram, ME1- MS + 2.5mg/L Picloram, MF1- MS+ 1.5mg/L Picloram, FW- Friable White, CW- Compact White, - No Callus, + Very poor callus, ++ Poor callus, +++ Good callus, ++++ Very good callus.
Source: Authors

leaves were washed with distilled water only without tween 20, it had no sterile leaf all the leaves were contaminated. This was also confirmed by Thomas and Puthur (2002) that in an experiment where tween 20 was deliberately omitted there was an increase in the percentage of contamination. Tween 20 is said to be a surfactant that act as a detergent which attaches itself to possible oil exudates helping to establish clean explants. The result of the present study on the effect of 2, 4 –D or Picloram on callus formation of Shea tree shows that basal media without plant growth regulator did not induce any callus growth and this was also observed by Dhiya et al. (2013) and Namrata et al. (2014) in the study on the influence of hormones and explants towards in vitro callusing and shoot organogenesis in a commercially important medicinal plant, callus was also not induced in media without growth regulators. The effect of different growth regulators on callus induction and on callus growth was the objective of this study. It was observed that to induce callus 2, 4-D medium was not effective enough whereas the callus remains healthy and increase in different concentration of picloram combination. The best callus initiation was observed with 2, 4-D in 1.5 mg/L, it showed friable callus. Thus the whole outcome showed that 2, 4 -D was not efficient for callus induction because of its toxic nature and also confirmed it that 2, 4-D is used as a form of herbicide to kill weeds. The result showed that when the concentration is high it kills the leaves.

The current result recorded that at 4.5 mg/L 2, 4-D and 4.5 mg/L picloram the callus induction percentage was low because of the increase in the concentration of the auxins. However, high concentrations of the Auxins are toxic to the plant cells. These facts explain the increase in the callus fresh weight as the concentration of the Auxins increased up to a specific level. High concentration of 2, 4-D (4. 5 mg/L) gave fewer calli than 1.5 mg/ L. The callus was watery and non regeneratible type. This might be due to Ethylene production which reduced cell division. Synthetic auxin like 2, 4-D has been developed as herbicide to control weed. The presence of growth regulators in culture medium is an important factor in

Table 5. Effect of MS media supplemented with different concentrations of picloram on callus formation from leaf explants of Shea tree at 4 weeks.

Growth regulators picloram	Callus induction percentage (%)	(Mean ± SE)	Degree of callus	Colour and morphology
Control	0	0	-	NC
MA2	35	1.75^{d}±0.25	+ +	FW
MB2	50	2.50^{c} ± 0.29	+ + +	FW
MC2	100	5.00^{a}±0.0	+ + + +	FW
MD2	80	4.00^{b}±0.41	+ + + +	FW
ME2	40	2.00^{cd}±0.0	+ + +	FW
MF2	35	1.75^{d}±0.25	+ +	FW

Mean with the same letters are not significantly different according to DMRT at p =0.05. MA1- MS + 4.5 mg/L Picloram, MB1- MS+ 4.0mg/L Picloram, MC1- MS+ 3.5 mg/L Picloram, MD1- MS+ 3.0 mg/L Picloram, ME1- MS + 2.5 mg/L Picloram, MF1- MS+ 1.5 mg/L Picloram, FW- Friable White, CW- Compact White, - No Callus, + Very poor callus, ++ Poor callus, +++ Good callus, ++++ Very good callus.
Source: Authors

callus proliferation, growth and differentiation of cultured cells (Kalidass et al., 2010). Liu et al. (2006) reported that 2, 4-D had been used singly and in combination with cytokinins to enhance callus induction and maintenance. In the present study, callus induction was observed after two weeks of inoculation. Adu-Gyamfi et al. (2012) also observed callus after the same duration though the explant used was cotyledon. Callus was initiated at the cut ends of the leaf explant of Shea. Callus formation at the proximal end of the explant in this study was in line with reports on *Pegannum harmla* (Saini and Jaiwal. 2002). The result of the present study on effect of picloram on callus induction 3.5 mg/L gave the best CI% of (100%) the callus is friable and creamy in appearance. According to Trigiano and Gray (2005) rapid growth and light colour callus usually indicate a healthy culture and a friable callus is a very suitable for breaking up either for sub culturing or to produce plantlets.

Conclusion

In this study, the best sterilization method of shea leaf explant was by soaking in 70% ethanol for 1 min followed by 15 min in 1% sodium hypochlorite with 1 µl of Tween 20. The optimum concentration for callus induction was 3.5 mg/L Picloram in MS media. *In-vitro* propagation of shea tree could serve as a viable alternative for raising woody plants with propagation difficulties. Application of appropriate hormones in the right proportion could also enhance tree growth response.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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REFERENCES

Adu-Gyamfi P, Barnor MT, Dadzie AM, Lowor SY, Opoku-Ameyaw K, Bissah M, Padi FK (2012). Preliminary investigation on somatic embryogenesis from immature cotyledson explants of Shea (*Vitellaria paradoxa* G.). Journal of Agricultural Science and Technology 12(4):1171-1176.

Amoo SO, Ayisire BE (2005). Induction of callus and somatic embryogenesis from cotyledon explants of *Parkia biglobosa.* African Journal of Biotechnology 4(6):548-553.

Anca P (2009). Biotechnology for endangered plant conservation: A critical overview. Romanian Biotechnological Letters 14(1):4095-4103.

Boffa JM (2015). Opportunities and challenges in the improvement of the Shea (*Vitellaria paradoxa*) resource and its management. Occasional Paper 24. Nairobi.

Dhiya ZD, Hafsah J, Manaf AA (2013). Effects of 2,4-D and Kinetin on Callus Induction of *Barringtonia racemosa* Leaf and Endosperm Explants in Different Types of Basal Media. Asian Journal of Plant Sciences 12(1):21-27.

Ghosh S (2005). Plant Tissue Culture and Its Application. New Central Book Agency Kolkata, India.

Kabir AH, Sarker KK, Sharmin SA, Islam MS, Alam MF (2008). Callus induction and plantlet regeneration in *Abelmoschus esculentus* (L.) Moench. Journal of Agricultural Technology 3(1):137-142.

Kalidass C, Mohan VR, Daniel A (2010). Effect of auxin and cytokinin on vincristine production by callus cultures of *Catharanthus roseus* L. (Apocyanaceae). Tropical and Subtropical Agroecosystems 12(1):283-288.

Khan SY, Zafar A, Yaseman K, Saeed B (2001). An efficient and economical method of mass multiplication of virus and disease free banana using plant tissue culture technique. Pakistan Journal of Biological Sciences 4(5):562-563.

Liu P, Zhang ZX, Yuan JG, Xi JB, Du XL, Yang ZY (2006). Callus induction and plant regeneration in eleven perennial ryegrass cultivars. Biotechnology and Biotechnological Equipment 20(4):30-37.

Lovett PN, Haq N (2013). Progress in developing in vitro systems for shea tree (*Vitellaria paradoxa* CF Gaertn.) propagation. Forests,

Trees and Livelihoods 22(1):60-69.
Maranz S, Wiesman Z (2003). Evidence for indigenous selection and distribution of the Shea Tree *Vitellaria paradoxa* and its potential significance to prevailing parkland savannah tree patterns in sub-Saharan Africa, north of the equator. Journal of Biogeography 30(10):1505-1516.
Mukherjee PA, Varshney TS, Johnson T, Jha TB (2011). Jatropha curcas: A review on biotechnological status and challenges. Plant Biotechnology Reports 5(3):197-215.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia plantarum 15(3):473-497.
Nalawade SM, Sagare AP, Lee CY, Kao CL, Tsy HS (2001). Studies on tissue culture of Chinese medicinal plant resources in Taiwan and their sustainable utilization. Botanical Bulletin of Academia Sinica 44(2):79-98.
Namrata SG, Maitreyi B, Krishnendu A (2014). Influence of hormones and explants towards in vitro callusing and shoot organogenesis in a commercially important medicinal plant. International Journal of Pharmaceutical Sciences Review and Research 29(2):132-137.
Nwala RC (2012). Somatic embryogenesis and plantlet regeneration from leaf explants of Shea tree (Vitelleria paradoxa) in the guinea savannah agro ecology of Nigeria. Source: Departmental library Crop Production FUTMINNA, Nigeria.
Cardoza N (2008). Tissue culture: The manipulation of plant development. Plant Biotechnology and Genetics: Principles, Techniques, and Applications pp. 113-134.
Srivastava LM (2002). Plant growth and development; hormones and environment Amsterdam. Academic press P 140.
Sharma V, Srivasta N, Kamal B, Dobriyal AK, Jadon SV (2014). Efficient sterilization protocols for different explants of an endangered medicinal herb *Swertia chirayita*. Trends in Life Sciences 3(1):2319-5037.
Thomas DT, Puthur JT (2004). Thidazuron induced higher fregeuncy shoot organogenesis in callus from *Kigelia pinnata L.* Botanical Bulletin of Academia Sinica 45(8):307-320.
Trigiano RN, Gray DJ (2005). Plant Tissue culture concepts and Laboratory Exercises. CRC Press LLC.
Yakubu FB, Asinwa IO, Shodeke DK, Williams OA, Obekpa NB (2015). Effects of NPK fertilizer on the shoot growth of *Vitellaria paradoxa* C.F. Gaertn. African Journal of Environmental Science and Technology 9(1):8-11.
Yeboah J, Akrofi AY, Owusu-Ansah F (2010). Influence of selected fungicides and hormones on the rooting success of Shea (*Vitelleria paradoxa* Gaertn) stem cuttings. Agriculture and Biology Journal of North America 1(3):313-320.