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Research Article

Explants selection for *in vitro* propagation of *Pachyrhizus erosus* L.

Idowu A. OBISESAN[1]*, Ayobola M.A. SAKPERE[2],
Bamidele J. AMUJOYEGBE[3], Michael S. AKINROPO[2]

[1]*Bowen University Iwo, College of Agriculture, Engineering and Sciences, Pure and Applied Biology, Osun State, Nigeria; idowu.obisesan@bowen.edu.ng (*corresponding author)*
[2]*Obafemi Awolowo University, Faculty of Science, Department of Botany, Ile-Ife, Osun State, Nigeria; aasakpere@oauife.edu.ng*
[3]*Obafemi Awolowo University, Faculty of Agriculture, Department of Crop production and Protection, Ile-Ife, Osun State, Nigeria; bamujo@oauife.edu.ng*

Abstract

Pachyrhizus erosus tuber is rich in protein asides its agronomical value as a legume, but the seeds by which it is propagated have very low viability. This study established sterilization protocol and effect of various concentrations of auxins and cytokinins on callus production and shoot regeneration from explants of *P. erosus*. Explants and seeds were sterilized using sodiumhypochlorite (NaClO) solution (5, 10 and 15% v/v) for 5 and 10 mins. Nodal, stem and leaf explants from *in vitro* germinated *P. erosus* and tuber from field grown plant were sterilized and cultured on Murashige and Skoog (MS) medium (control) and MS combined with different concentrations of auxins (NAA and 2, 4-D) and cytokinin (BA and Kinetin) and the cultured explants were monitored in terms of degree of callus formation, morphology and colour of callus and also for shoot induction. The results showed that seeds of *P. erosus* sterilized with 10% NaClO solution for 10 mins and germinated *in vitro* is the best way of getting sterile nodal, stem and leaf explants for the *in vitro* propagation of the plant, while tuber explants could be sterilized with 15% NaClO for 10 minutes. Nodal explants inoculated in MS medium supplemented with 1.0 mg/L BA gave the highest shoot regeneration response, while stem explants inoculated on MS medium supplemented with 1.0 mg/L BA and 0.5 mg/L NAA also gave the highest amount of friable callus. The study concluded that *in vitro* germinated seeds were the best way of getting explant for *P. erosus*.

Keywords: auxins; callus; contamination; cytokinins; legume; *Pachyrhizus erosus*; shoot regeneration

Introduction

Food security, protein malnutrition, increasing population, uncertain crop yield and high cost of animal-based protein food supplies in developing countries have created an urge to identify and incorporate unconventional protein source to supplement indigenous crop (Masood and Rizwana, 2010). Diversifying diets with legumes are a cheaper and more sustainable way to supply a range of nutrients to the body and combat malnutrition. Therefore, the need for innovative legume research solutions to improve food and nutritional security cannot be overemphasized (Ojiewo *et al.*, 2015).

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Pachyrhizus erosus commonly called Mexican yam bean is an important legume with high nutritional value. Its tubers contain higher protein than most African indigenous tubers (Santos *et al.*, 1996; Bergthaller *et al.*, 2001; Slavin, 2005; Padonou, 2013). Propagation of *P. erosus* is by seeds which are known to have rapid decrease in germination after 4 hours and remained viable for only 22 hours (Sorensen, 1996), giving rise to decreasing seed viability during storage. An alternative means of propagation for mass cultivation therefore necessitate its micropropagation. *In vitro* plant tissue culture has been reported to be a good alternative for the propagation and conservation of economic legumes. For instance, Singh *et al.* (2019) established a protocol for *in vitro* propagation of the wild legume *Cicer microphyllum*. Shahinozzaman *et al.* (2012) established a protocol for *in vitro* clonal propagation of *Acacia mangium* using seedling derived explants.

Explants contamination is one of the major problems encountered during propagation of plants *in vitro*, especially from underground organs (such as tubers), which has been in contact with debris and soil microorganisms (Cassells, 1991; Omamor *et al.*, 2007; Singh *et al.*, 2011). Varying the concentration of sterilant and duration of contact of explants with sterilants has been in practice amongst *in vitro* researches (Niedz *et al.*, 2002; Mahna *et al.*, 2013). Determining the best explants for callus formation and multiple shoot regeneration is important in establishing a protocol for micropropagation of the plant.

The objective of this study was to investigate the best sterilization method for *in vitro* propagation of *P. erosus*, to determine the best explants for *in vitro* propagation of the plant; and study the effect of different concentrations of hormones on regeneration ability and callus formation in the plant.

The study provides information on the micropropagation of *P. erosus* as an alternative method to mitigate the effect of loss of seed viability and for rapid multiplication of the plant as well as for possible future genetic modification of *P. erosus*.

Materials and Methods

Media preparations and culture conditions

Murashige and Skoog (1962) (MS) media was used for the study. This was supplemented with 7.5 g sucrose, 0.025 g myo-inositol, 0.0093 g EDTA, 0.0069 g Fe, and made up to mark with distilled water (Sathyanarayana and Verghese, 2007). The solution was poured into a beaker and the pH adjusted to 5.7 ± 0.2 with 0.1 N (Normal) HCl and/or 0.1N NaOH before adding 0.5 g phytagel as the gelling agent. The medium was thereafter heated on a hot plate while mixing with a magnetic stirrer until it boiled, after which 5 ml each of the medium was dispensed into 50 ml test tubes. The test tubes were covered with cotton-wool, wrapped with parafilm and autoclaved for 15 minutes (min) at 121 °C and 108 K Pa.

Surface sterilization procedure for explants

Four weeks old stem, node, leaf and 12 weeks old tuber collected from screen house were used as sources of explants. Five replicates were used for each explant and the experiment carried out twice. Sterilant (Chlorox ©) which contains 8.25% NaClO solution was used at 5% v/v, 10% v/v and 15% v/v for 5 min and 10 min each respectively.

Explants were cut into approximately 1cm each and were washed with liquid soap and rinsed under running tap for 30 min. The explants were transferred under a Laminar Flow Hood (under sterile condition) into 70% ethanol for 5 min. The ethanol was decanted and the explants further sterilized in NaClO solution for the different specified percentages and durations earlier mentioned, with 2 drops of Tween 20 added. After sterilization, the explants were rinsed with sterile distilled water thrice. The explants were cultured in 5 ml MS media in 50 ml capacity test tubes and then transferred to the growth room maintained at 25 °C ± 2 °C and 16 hours light and 8 hours darkness photoperiod.

Sixty *P. erosus* seeds were soaked for 9 hours (over-night) to soften the testa. Seed testa were removed with the aid of forceps, the cotyledon and intact embryo washed with liquid soap and rinsed under running

water for 15 min. These were transferred into 250 ml of 70% ethanol for 5 minutes in a Laminar Flow Hood. After decanting the ethanol, the seeds were transferred into NaClO solution with two drops of Tween 20 for the different specified percentages and durations mentioned above. Thereafter, seeds were rinsed thrice with sterile distilled water before inoculation into 5 ml of MS media in test tubes.

Direct regeneration and callus initiation from P. erosus explants

Different concentrations of plant growth regulator (Table 1) were supplemented in MS medium and tested for their effects on callus induction and shoot regeneration from explants of field grown tuber, and also from nodal, leaf and stem explants excised from *in vitro* germinated seedlings. Explants were cultured on MS medium alone as control (Treatment 1); five explants were used per treatment. The experiment was carried out twice and was observed within the period of 4 weeks.

Table 1. Concentrations and combinations of BA, NAA, kinetin and 2,4-D used as supplements in MS medium for investigating callus induction and shoot regeneration from *P. erosus* explants

Treatment	BA (mg/L)	NAA (mg/L)	Kinetin (mg/L)	2,4-D (mg/L)
1	-	-	-	-
2	1.0	-	-	-
3	1.0	0.5	-	-
4	-	0.5	-	-
5	-	-	0.5	0.25
6	-	-	0.75	0.5
7	-	-	1.0	0.75
8	-	0.25	0.5	-
9	-	0.5	0.75	-
10	-	0.75	1.0	-
11	-	-	0.5	-
12	-	-	0.75	-
13	-	-	1.0	-

Statistical analysis

Data collection was based on visual observation of single and multiple shoot induction, also on degree and morphology of callus. All data were analysed by calculating mean ± standard error using analysis of variance by Generalized Linear Model of SAS 9.2 version. Means were separated using Duncan's multiple range tests at 0.05 probability level.

Results

Surface sterilization for explants of P. erosus

Stem and leaf explants sterilized with 5 and 10% v/v NaClO at both 5 and 10 mins, were contaminated and 15% v/v surface sterilized explants at both durations were observed dead a week after inoculation in MS media (Figure 1).

Nodal explants sterilized with 10% v/v NaClO solution at both 5 min (20%) and 10 min (30%) regenerated clean shoots two weeks after inoculation in MS media. While nodal explants sterilized with 5% v/v and 15% v/v NaClO solutions were contaminated and dead respectively, with no shoot formation (Figure 1).

Root tuber explants sterilized with 15% v/v NaClO solution for 10 min were observed clean (50%) with callus formation. While the other treatments (5% and 10%) became contaminated without callus formed, one week after inoculation (Figure 1).

P. erosus seeds sterilized with 5% v/v NaClO for 5 and 10 min. germinated with 40% and 30% clean seedlings respectively after two weeks. Seeds sterilized with 10% v/v NaClO solution for 5 min germinated with 40% of clean seedlings. Seeds sterilized with 10% v/v NaClO solution for 10 min had the highest percentage of clean seedlings (90%) (Figure 1).

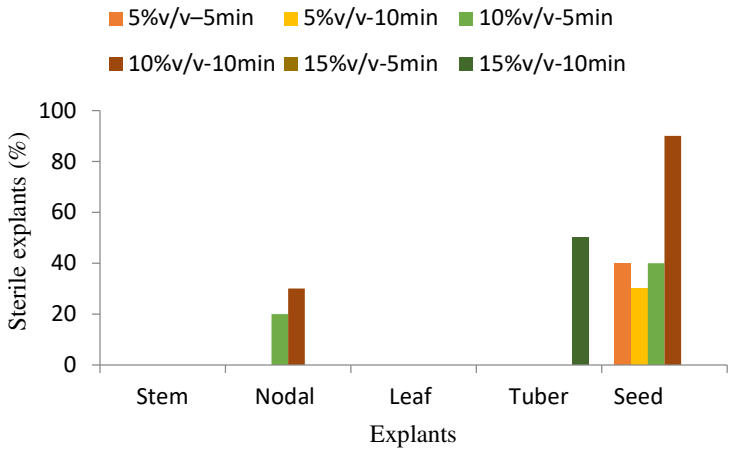

Figure 1. Effect of different concentrations of NaClO solution on surface sterilization of *P. erosus* explants (n=10)

Direct regeneration and callus formation on nodal explants of P. erosus

P. erosus nodal explant cultured in MS medium supplemented with 1.0 mg/L BA only and in MS medium supplemented with 1.0 mg/L BA + 0.5 mg/L NAA gave the significantly highest number of multiple shoots and also gave a significantly higher degree of friable callus compare to other treatments (Table 2 and Figure 2A&B). The different concentrations of kinetin and 2, 4-D supplements in MS media also gave considerable number of multiple shoots, it produced moderate callus that are compact in nature or no callus production at all (Table 2 and Figure 2C).

Table 2. Effect of different concentrations of auxins (NAA and 2, 4-D) and cytokinins (BA and kinetins) on shoot induction and callus formation from nodal explants of *in vitro* germinated *P. erosus*

Treatment (mg/L)	Quantity of shoot	No. of shoot induced per explant	Mean no. of shoots ±S. E	Callus formation (Mean± S.E)	Degree of callus	Morphology of callus formation
MS (Control)	S	1.33 ± 0.33^{d}	0.40 ± 0.02^{cd}	0.00 ± 0.00	-	-
MS + 1.0 BA	M	4.00 ± 0.00^{a}	6.80 ± 0.11^{a}	4.00 ± 0.00^{a}	++	Friable
MS+1.0 BA+ 0.5 NAA	M	3.00 ± 0.00^{ab}	3.40 ± 0.20^{b}	3.00 ± 0.00^{ab}	++	Friable
MS+0.5 NAA	M	2.33 ± 0.33^{bcd}	1.26 ± 0.12^{bc}	0.00 ± 0.00	-	-
MS+0.5 Kin + 0.25 2,4-D	S	2.33 ± 0.33^{bcd}	1.20 ± 0.14^{bc}	2.33 ± 0.33^{bcd}	++	Compact
MS+0.75 Kin + 0.5 2,4-D	S	1.67 ± 0.33^{cd}	0.52 ± 0.34^{c}	1.67 ± 0.33^{cd}	++	Compact
MS+1.0 Kin+0.75 2,4-D	-	0.00 ± 0.00	0.00 ± 0.00	2.67 ± 0.33^{bc}	++	Compact
MS+0.25 NAA+0.5Kin.	-	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.57^{bcd}	++	Compact
MS+0.5NAA+ 0.75Kin	-	0.00 ± 0.00	0.00 ± 0.00	1.67 ± 0.33^{cd}	++	Compact
MS+0.75NAA+1.0Kin	S	2.67 ± 0.33^{bc}	1.00 ± 0.10^{c}	1.67 ± 0.33^{cd}	++	Compact
MS+0.5Kin	S	1.33 ± 0.33^{d}	0.60 ± 0.42^{c}	1.33 ± 0.33^{d}	++	Compact
MS+0.75Kin	M	2.67 ± 0.33^{bc}	2.40 ± 0.31^{b}	0.00 ± 0.00	-	-
MS+1.0Kin	M	2.81 ± 0.33^{bc}	2.80 ± 0.42^{b}	1.68 ± 0.57^{bcd}	++	Compact

Values with the same superscript in a column are not significantly different from each other at P≤ 0.05. (n=10). S (Single Shoot), M (Multiple Shoots). (+ Scanty callus), (++ Moderate callus), (+++ Massive callus)

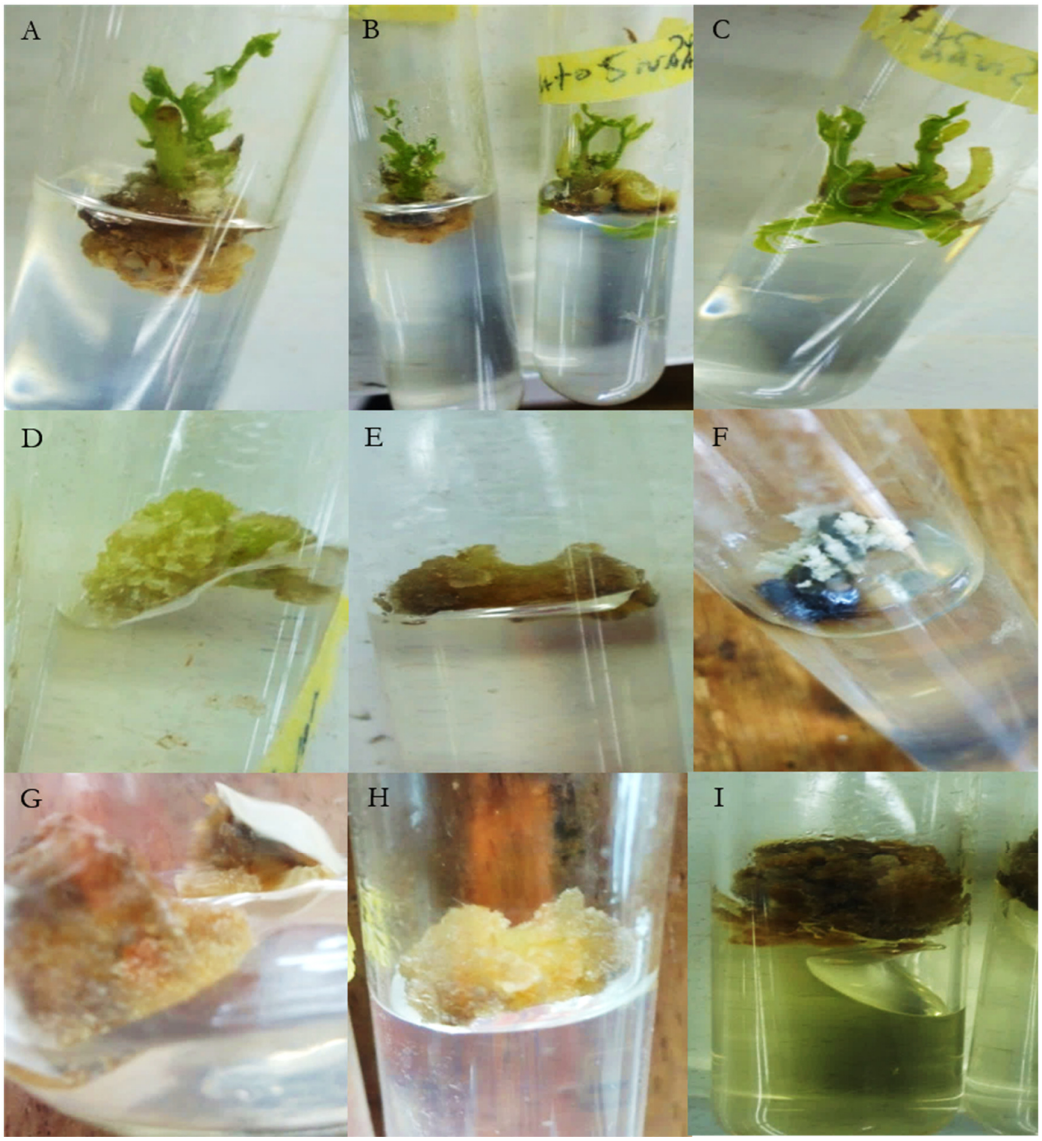

Figure 2(A-I). Shoot and callus formation of *in vivo* explants of *P. erosus* 4 weeks after inoculation on MS media + PGR

(A) Nodal explants with multiple shoots and basal callus on MS media + 1.0 BA mg/L (B) Nodal explants with multiple shoots and basal callus on MS media + 1.0 mg/L BA + 0.5 mg/L NAA (C) Nodal explants with multiple shoots on MS media + 0.5 mg/L NAA. (D) Stem explants forming friable callus on MS media + 1.0 mg/L BA + 0.5 mg/L NAA. (E) Stem explants forming friable callus on MS media + 0.5 mg/L NAA (F) Stem explants with compact callus on MS media + 0.75 mg/L NAA+ 1.0 mg/L Kin. (G) Tuber explants with friable callus on MS media + 1.0 mg/L BA + 0.5 mg/L NAA. (H) Tuber explants with friable callus on MS media + 1.0 mg/L BA only (I) Tuber explants with compact callus and phenolic exudates on MS media + 1.0 mg/L Kin

Direct regeneration and callus formation on stem explant of P. erosus

Stem explants from *In vitro* germinated *P. erosus* cultured in MS media supplemented with different concentrations of auxins and cytokinins did not produce any shoot, but callus was formed. Stem explants cultured in MS medium supplemented with 1.0 mg/L BA + 0.5 mg/L NAA and in MS media supplemented

in 0.5 mg/L NAA only, gave the significantly highest friable callus production ($P < 0.05$) (Table 3 and Figure 2 D, E and F).

Table 3. Effect of different concentrations of auxins (NAA and 2, 4-D) and cytokinins (BA and kinetins) on callus formation from stem explants of *in vitro* germinated *P. erosus*

Treatment (mg/L)	Callus formation (Mean ± S.E)	Degree of callus	Morphology of callus
MS (Control)	0.00 ± 0.00^{d}	-	-
MS + 1.0 BA	2.67 ± 0.67^{bc}	++	Friable
MS+1.0 BA+ 0.5 NAA	4.33 ± 0.33^{a}	++	Friable
MS+0.5 NAA	3.67 ± 0.67^{ab}	++	Friable
MS+0.5 Kin + 0.25 2,4-D	1.33 ± 0.33^{cd}	++	Friable
MS+0.75 Kin + 0.5 2,4-D	1.67 ± 0.33^{c}	+	Friable
MS+1.0 Kin+0.75 2,4-D	1.00 ± 0.00^{cd}	++	Friable
MS+0.25 NAA+0.5Kin.	2.33 ± 0.33^{bc}	++	Compact
MS+0.5NAA+ 0.75Kin	2.33 ± 0.44^{bc}	++	Compact
MS+0.75NAA+1.0Kin	3.33 ± 0.33^{ab}	+++	Compact
MS+0.5Kin	2.33 ± 0.67^{bc}	+++	Compact
MS+0.75Kin	2.33 ± 0.88^{bc}	++	Compact
MS+1.0Kin	2.67 ± 0.67^{bc}	+	Compact

Values with the same superscript in column are not significantly different from each other at $P\leq 0.05$. (n=10). (+ Scanty callus), (++ Moderate callus), (+++ Massive callus)

Direct regeneration and callus formation on tuber explant of P. erosus

Friable callus was observed on tuber explant cultured on MS media supplemented with 1.0 mg/L BA + 0.5 mg/L NAA, 1.0 mg/L BA alone and 0.5 mg/L NAA alone. Compact callus with brownish exudates were observed on tuber explants cultured on MS media supplemented with 1.0 mg/L Kin alone, 0.75 mg/L Kin alone, 0.25 mg/L NAA + 0.5 mg/L Kin and 0.75 mg/L NAA + 1.0 mg/L Kin (Table 4).

Callus formation was significantly highest in tuber explants cultured on MS medium supplemented with 1.0 mg/L BA + 0.5 mg/L NAA (Figure 2G), but not significantly higher than callus produced on tuber explants cultured on media supplemented with 1.0 mg/L Kin alone (Figure 2 I) and 1.0 mg/L BA alone (Figure 2 H) ($P<0.05$)(Table 4).

Table 4. Effect of different concentrations of auxins (NAA and 2, 4-D) and cytokinins (BA and kinetins) on callus formation from tuber explants of *P. erosus*

Treatment (mg/L)	Callus formation (Mean ± S.E)	Degree of callus	Morphology of callus
MS (Control)	0.00±0.00	-	-
MS + 1.0 BA	3.00 ± 0.00^{abc}	+++	Friable
MS+1.0 BA+ 0.5 NAA	4.00 ± 0.57^{a}	+++	Friable
MS+0.5 NAA	2.00 ± 1.00^{bcd}	++	Friable
MS+0.5 Kin + 0.25 2,4-D	1.33 ± 0.33^{def}	++	Compact
MS+0.75 Kin + 0.5 2,4-D	1.33 ± 0.33^{def}	++	Compact
MS+1.0 Kin+0.75 2,4-D	0.33 ± 0.33^{ef}	++	Compact
MS+0.25 NAA+0.5Kin.	1.67 ± 0.33^{cde}	++	Compact
MS+0.5NAA+ 0.75Kin	2.33 ± 0.33^{bcd}	++	Compact
MS+0.75NAA+1.0Kin	1.33 ± 0.33^{def}	+	Compact
MS+0.5Kin	1.00 ± 0.57^{def}	++	Compact
MS+0.75Kin	2.33 ± 0.33^{bcd}	++	Compact

MS+1.0Kin	3.33±0.33^{ab}	++	Compact

Values with the same superscript in column are not significantly different from each other at P≤ 0.05. (n=10) (+ Scanty callus), (++ Moderate callus), (+++ Massive callus)

Direct regeneration and callus formation on leaf explant of P. erosus

There were no shoot or callus formation on leaf explants of *P. erosus* inoculated on MS basal medium and on MS media supplemented with various concentrations of BA or Kinetin alone or in combinations with 2, 4-D and NAA four weeks after inoculation.

Effect of different concentrations of hormones on shoot formation in different explants of P. erosus

Multiple and single shoots were formed from nodal explants of *P. erosus* for the various concentrations of BA or Kinetin alone or in combinations with 2, 4-D and NAA four weeks after inoculation. Shoot formation was suppressed on nodal explants inoculated on MS media supplemented with higher concentrations of Kinetin in combination with NAA or 2,4-D. There was no shoot formed on stem, tuber and leaf explants for the various concentrations of BA or Kinetin alone or in combinations with 2, 4-D and NAA four weeks after inoculation (Figure 3).

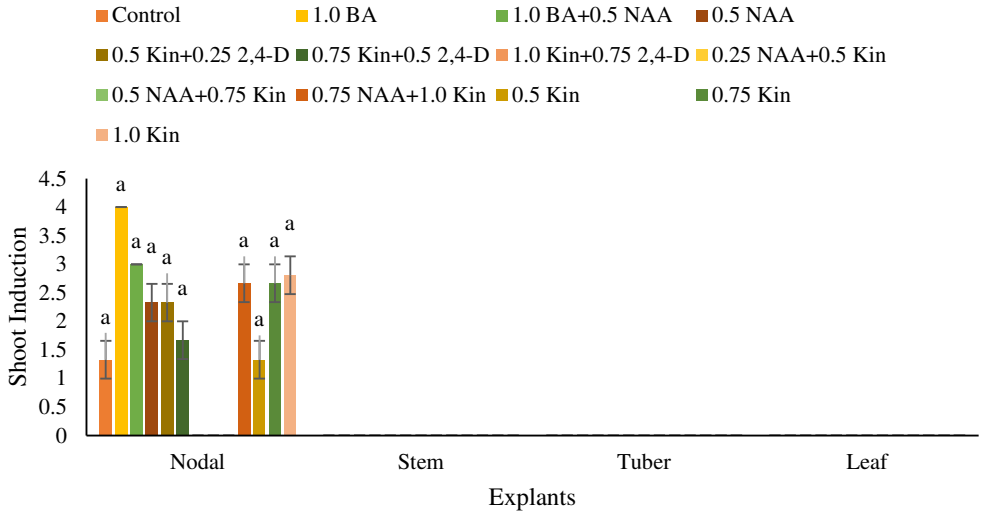

Figure 3. Effect of different concentrations of auxins (NAA and 2, 4-D) and cytokinins (BA and kinetins) on shoot formation in different explants of *P. erosus*

Values with the same label across different plant organs (explants) are not significantly different from each other at P≤ 0.05. (n=10)

Effect of different concentrations of hormones on callus formation in different explants of P. erosus

Callus formation was significantly higher in nodal explants inoculated on MS medium supplemented with 1.0 mg/L BA than in other explants, except for tuber explant in which there was no significant difference (P≤0.05). Stem and tuber explants inoculated on media supplemented with 1.0 mg/L BA + 0.5 mg/L NAA gave a high quantity of callus but not significantly higher (P≤0.05) than that of nodal explants. Callus formation was significantly higher (P≤0.05) in stem explant inoculated on media supplemented with 0.5 mg/L NAA than in tuber, while there was no callus initiation on nodal and leaf explant. Callus initiated on nodal, stem and tuber explants inoculated on MS media supplemented with 0.5 mg/L Kin. + 0.24 mg/L 2,4-D, 0.75 mg/L Kin + 0.5 mg/L 2,4-D and 1.0 Kin + 0.75 2,4–D Kin were not significantly different from one another (P≤0.05). Callus formation on nodal, stem and tuber explants inoculated on MS media supplemented with 0.25 mg/L NAA + 0.5 mg/L Kin, 0.5 mg/L NAA + 0.75 mg/L Kin and 0.75 mg/L NAA + 1.0 mg/L Kin were not significantly different from one another (P≤0.05). Callus formation was more on stem explants than in

tuber explants inoculated on MS media supplemented with 0.5 mg/L Kin, but was not significantly different (P≤0.05) from callus formed on the nodal explants. Callus formation was more on tuber explants than on nodal explants inoculated on MS media supplemented with 1.0 mg/L Kin, but was not significantly different from callus formed on the stem explants (P≤0.05) (Figure 4).

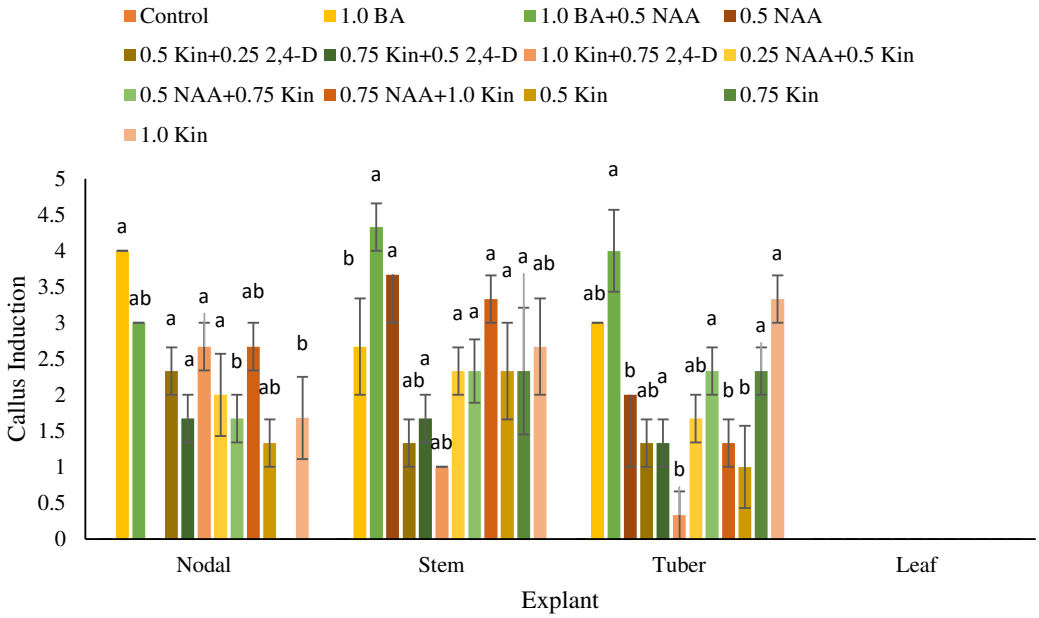

Figure 4. Effect of different concentrations of auxins (NAA and 2, 4-D) and cytokinins (BA and kinetins) on callus formation in different explants of *P. erosus*

Values with the same label across different plant organs (explants) are not significantly different from each other at P≤ 0.05. (n=10)

Discussion

Surface sterilization of P. erosus explants

Contaminations in culture media could be introduced from many sources such as pathogen or microbial infections on plant materials collected from the field, contaminations from reagents, unsterilized laboratory equipment, improper handling of culture media etc. These could promote growth of microorganisms on cultured media. Surface sterilization of explants from field grown plants intended for *in vitro* culture is therefore of utmost importance. The percentage contamination of *P. erosus* explants (nodal, leaf and stem) obtained from the plants grown in the field in this study was very high, for the different concentrations of NaClO solutions used in surface sterilization at different durations. This could be as a result of plant pathogens present in the environment where the plants were cultivated. Some of which can be insect infestations which bring in fungal and bacterial contamination (Smith, 2008). Stems and leaves of *P. erosus* cultivated in the field were observed to have epidermal hairs (i.e. pubescent), which will be a good anchorage for microorganisms causing contaminations (Marvasi *et al.*, 2012). Surfaces covered with thick wax such as the succulents and epidermal hairs can also trap microorganisms. Additionally, many plants have microbial contamination within the vascular system, and intercellular spaces in leaf mesophyll (Miyazaki *et al.*, 2013). Legumes particularly have been reported to be highly susceptible to insect pests and diseases which causes significant damage to the plant in the field (Srinivasan and Yule, 2014), of which, *P. erosus*, might not be an exception. A higher concentration

of NaClO solutions (15%) was noted to be too strong on the tissues of the nodal, stem and leaf explants, resulting in death of the explants. This shows that higher concentration of the sterilant is toxic to the explants. On the other hand, lower concentration of NaClO were found to be ineffective in sterilization of tuber explants, while a higher concentration gave an average response. This is because maintaining an aseptic condition for plant organs that have been in contact with the soil particles which usually contains microorganisms is highly tasking. This observation is supported by the report of Bunn and Tan (2002), which stated that plant material growing in the soil (roots, tubers, bulbs) or near the soil surface (stolons, rhizomes, shoots from rosettes) is usually more difficult to clean than aerial plant part. Tuber explants, therefore, require extra care, like peeling the skin off the edible tuber to remove the part of the explant that has been in direct contact with the soil and microorganisms in the soil, before surface sterilization. The tuber explants were able to survive the higher concentration (15% NaClO) of the sterilant being hardier unlike the nodal, stem and particularly the leaf explants, which could not survive the higher concentration of NaClO solution.

An alternative way of getting explants for *in vitro* culture is through *in vitro* germination of seeds. Miransari and Smith (2014) suggested that seeds can be aseptically germinated to provide clean explants from the root, hypocotyl, cotyledon, and shoot. *P. erosus* aseptically germinated *in vitro* were observed to develop stems, leaves and nodes that are without epidermal hairs (i.e. glabrous). This eliminated the possibility of microbial infections caused by pathogens carried by trichomes on the field grown plant organs. This observation is supported by the research of Lochlainn (2011) in which *P. erosus* seeds were germinated *in vitro* for the purpose of micro propagation. Kumar and Chandra (2009) also reported that i*n vitro* grown seedlings can be used as the starting plant material for tissue culture because they can be quickly raised and chances of bacterial and fungal contaminations in the tissue cultures can be minimized. In this study, seeds sterilized with 10% NaClO solution for 10 minutes and germinated *in vitro* were discovered to be the best way of getting sterile nodal, stem and leaf explants for the *in vitro* propagation of *P. erosus.* While tuber explants sterilized with 15% NaClO for 10 minutes also gave an average response.

Effect of different hormones on direct regeneration and callus formation in P. erosus

Nodal explants inoculated on MS basal media only had single shoot inductions. This was because nodal explants have been reported to have a great organogenic potential for direct shoot regeneration (Neves *et al.*, 2001; Shaik*et al.*, 2010; Ayisire *et al.*, 2012). Although, nodal explants on media supplemented with 1.0 mg/L BA alone and 1.0 BA and 0.5 mg/L NAA produced multiple shoots with friable callus, those on media supplemented with 1.0 mg/L Kin alone and 0.75 Kin alone produced multiple shoots with compact callus. This is supported by Shagufta *et al.* (2007) in which BA was reported to be an ideal hormone for shoot multiplication in grain legumes. Shaik*et al.* (2010) also reported that the most effective cytokinin for shoot multiplication of *Lessertia frutescens* (a member of the family Fabaceae) using nodal explants was BA. Kumar and Chandra (2009) observed a considerable increase in the size of explant followed by multiple shoot induction within 4-6 weeks of incubation of *Stylosanthes seabrana*. The addition of BA alone and Kinetin alone in the medium resulted in the induction of multiple shoots. Neves *et al.* (2001) also used cytokinin for multiple shoot induction in *Medicago truncatula* and reported BA to be the best for inducing maximum number of shoots. The two cytokinins used in this study, BA and Kinetin gave multiple shoot induction, even though the amount of multiple shoots induced was significantly lower in Kinetin compared to BA. In this study, the combination of 1.0 mg/L BA and 0.5 mg/L NAA supplemented in MS medium produced multiple shoots from nodal explants. However, addition of NAA in the medium reduced the regeneration efficiency of the nodal explants. This is contrary to the study reported by Rout (2005) that *in vitro* shoot multiplication from nodal explants of *Clitoria ternatea* on MS basal medium supplemented with BA with inclusion of NAA promoted higher rates of shoot multiplication than BA alone. Lochlainn (2011) observed a contrary report of nodal explants inoculated on MS media supplemented with NAA and BA producing only callus.

Stem, leaf and tuber explants of *P. erosus* in this study did not produce shoots in the MS media supplemented with different concentrations of auxins and cytokinin. The inability of these explants to produce

shoot might be because of their morphology, developmental stage and/or choice and concentration of hormones used. It could also be because different explants and even species respond differently to the same treatment (Sakpere *et al.*, 2011).

Production of callus from fragments of stems, leaves and roots are mainly carried out to determine the culture conditions required by the explants to survive and grow, study cell development, exploit products coming from primary and secondary metabolism and obtain cell suspension in propagation. It can also pave the way for isolating economically valuable phytochemicals, which can provide an alternative way of collecting plant materials from natural sources (Berkov *et al.*, 2009; Ogita *et al.*, 2009). In the areas of plant biotechnology, callus and cell culture carries a special role for producing medicinal and bioactive compounds in large-scale from plants (Monokesh, 2014). Phytochemicals are serving as a major source of pharmaceuticals, flavors, agrochemicals, colors, bio-pesticides and food additives. Cytokinins have been reported to play an important role in callogenesis (He *et al.,* 2002) while auxin affects the quality of callus formation (Chen *et al.,* 2002). In this study, high response of friable callus which could be further used for suspension culture for the purpose of embryogenesis, were produced from nodal, stem and tuber explants inoculated in MS medium supplemented with 1.0 mg/L BA alone and in MS medium supplemented with 1.0 mg/L BA plus 0.5 mg/L NAA. While nodal, stem and tuber explants inoculated on MS medium supplemented with Kin alone, combination of Kin with 2, 4-D and combination of NAA with Kin produced compact callus which could not be used for suspension culture. However, for stem explants addition of 2, 4-D to Kin. induced friable callus formation. Gupta *et al.* (2005) also obtained callus from stem and root segments of *Boerhaavia diffusa* plantlets produced *in vitro* on MS medium supplemented with 0.5 mg/L BA and 1.0 mg/L NAA. Although, in this research, stem explants gave a relatively higher response of friable callus compared to nodal and tuber explants. However, leaf explants inoculated in MS medium alone and in MS medium supplemented with different concentrations and combinations of BA, NAA, Kin and 2, 4 – D, where unable to produce callus.

Conclusions

In determining the best explants for *in vitro* propagation of *P. erosus*, nodal explants were discovered to be the best for shoot regeneration. Stem, tuber and nodal explants are callogenic and are good sources of callus that could be used for production of secondary metabolites and genetic transformation studies, with stem explants having higher callus induction capacity.

Authors' Contributions

OIA, SAMA and ABJ conceived the study design. OIA and AMS carried out all experiments. OIA drafted the first manuscript. OIA and SAMA improved the manuscripts. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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