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Full Length Research Article

STUDIES ON THE EFFECTS OF 6-BENZYLAMINOPURINE AND 1-NAPHTHALENEACETIC ACID ON THE *IN VITRO* REGENERATION OF OKRA (*ABELMOSCHUS ESCULENTUS* L.)

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ABSTRACT
Okra is an important vegetable crop which belongs to the family Malvaceae. Experiments were created to study the effects of varying concentrations of Plant Growth Regulators (PGRs) on the *in vitro* propagation of okra using seed explants. The seeds were cultured *in vitro* on Murashige and Skoog (MS) basal media containing 30% sucrose, 0.9% agar and different concentrations (0.1mg/L to 0.25mg/L) of auxin (NAA) and cytokinin (BAP) singly and in combination. Early germination was obtained at 0.25mg/L BAP and 0.25mg/L NAA. Highest height was recorded at 0.15mg/L (12.8cm) followed by a combination of 0.25mg/L BAP and 0.25mg/L NAA (4.91cm). 0.15mg/L NAA followed by a combination of 0.25mg/L NAA and 0.25mg/L BAP and 0.1mg/L BAP and 0.1mg/L NAA in combination gave best vigor. Similarly, full strength media supplemented with 0.15mg/L NAA followed by 0.25mg/L BAP and 0.25mg/L NAA and 0.1mg/L BAP and 0.1mg/L NAA in combination gave the highest number of leaves. Similarly, 0.1mg/L NAA and a combination treatments of 0.1mg/L BAP and 0.1mg/L NAA with 0.25mg/L BAP and 0.25mg/L NAA gave the best percentage germination at 25%. Results of Analysis Of Variance (ANOVA) indicated significant differences among the treatments compared with the control which did not respond $p < 0.05$. The protocol developed in this present study can be used for large scale seedling formation and biomass production of okra. It can also be used to obtain sterile and uniform materials for various *in vitro* studies for the improvement of okra.

Keywords: Plant Growth Regulators, *in vitro*, okra, explants.

INTRODUCTION

Okra (*Abelmoschus esculentus* L.) belongs to the family *Malvaceae* (Thompson and Kelly, 1957). It is ubiquitous in distribution and grown in all parts of the tropics during summer in the warmer parts of the temperate regions (Baloch, 1994). Presently, okra is grown in an area of around 0.78 million hectares world-wide with an annual production of 4.9 million metric tonnes in the year 2005 (Singh, 1990; FAO Statistical Database, 2005). It is a good source of vitamin A and vitamin C. It is low in calories and is fat free. It has also considerable medicinal and industrial value (Kirtiker and Basu, 1984). It is diploid with 2n = 130 chromosomes, semi woody, fibrous, herbaceous annual crop with an indeterminate growth habit. The plant form a deeply penetrating taproot with dense shallow feeder roots reaching out in all directions in the upper 45 cm of the soil. The leaves are heart-shaped and tri or penta lobed, flowers are yellow with a crimson centre. The fruit or pod is tapering and hairy at the base, 10-25 cm in length that contains numerous oval dark coloured seeds, and only the tender unripe fruits are eaten. The seeds are dicotyledonous and kidney shaped exhibiting an epigeal type of germination (Nonnecke, 1989). The fruit is useful in curing ulcers, haemorrhoids, gonorrhoea and irritation of the urinary genital system (Kirtikar and Basu, 1984). Therefore, it is necessary to develop an effective regeneration protocol by a range of different techniques to widen the possibilities for the development of transgenic lines or somaclonal variants of different cultivars (Chopra *et al*., 1986).

Processes of plant growth require the action and cross talk of phytohormones such as auxins and cytokinins (Bajguz and Piotrowska, 2009). By setting phytohormone concentration in the medium, differences in amount, rate and growth patterns of explants can be observed (Pierik, 1987). Many members of the *Malvaceae* have been rather recalcitrant due to poor seed germination, endogenous fungal and bacterial contamination and low viability of seeds (Salunke *et al*., 1988).

Ethylene, a naturally occurring substance, is one of the first plant growth regulators being discovered and used successfully for enhancing flower production in pineapple. Synthetic substances that mimic such naturally occurring plant hormones are also produced, since then the use of plant growth regulators has been growing significantly and becoming a major component in modern agriculture (Fishel, 2006).

Plant tissue culture techniques have been widely used for micro propagation of plants since the early demonstration of cellular totipotency and differentiation *in vitro* (Murashige 1974). *In vitro* culture of okra will produce a large number of genetically identical individuals as well as a rapid propagation of this important horticultural crop. (Vasil and Vasil, 1972; Thorpe, 2007). This technique can also be implored in the conservation of germplasm of rare species of okra plant that are not common in Nigeria (Dhankar *et al*., 2013). The improvement of the micro-propagation system for this species could be important for its commercial production (Vargas *et al*., 2004).A few previous works have been done *in vitro* for okra (Haider *et al*., 1993; Ganesan *et al*., 2007). The ability to regenerate okra plants *in vitro* would permit the

The aim of this work is to study the effects of varying concentrations of BAP and NAA Plant Growth Regulators (PGRs) on the *in vitro* propagation of okra.

MATERIALS AND METHODS

Study Area

The experiments were conducted in the Biotechnology Laboratory of Plant Science Department, Ahmadu Bello University, Zaria, located on latitude 11°11 N and longitude 07°38E, altitude 670m above mean sea level, 640km from the Atlantic shores of Nigeria in the south and transported to the Post Graduate (PG) laboratory

of Biological Science Department, Kaduna State University (KASU) located on Latitude10.520 N and 7.440 E Longitude, 614m elevation above sea level, Tafawa Balewa way, Kaduna metropolis, Kaduna, Nigeria for observation and data recording.

Source of Explants

Seeds of Okra (*Abelmoschus esculentus* L.) were collected from Kaduna central market, Kaduna State, Nigeria within the northern Guinea Savanna.

Experimental design

Experiment was carried out using Completely Randomize Design (CRD). Individually each experiment was replicated 2 times.

Sterilization of explants

i. All the seeds explants were soaked in hot water respectively for 2 minutes before they were surface sterilized.
ii. Seeds were washed outside the laminar flow hood with household detergent and rinsed with running tap water.
iii. Seeds were washed again with detergent and rinsed with double distilled water in laminar flow hood.
iv. Seeds were poured again into 70% ethanol for 3minutes and rinsed with double distilled water.
v. Seeds were then soaked in 30% sodium hypochlorite for 10 minutes. After this treatment, the seeds were rinsed 5 times in sterile distilled water to make free the seeds from sodium hypochlorite.
vi. Sterilized seeds were then subjected to aseptic conditions so as to germinate in bama (media) bottles containing 50ml full strength MS (Murashige and Skoog, 1962) basal media (Hamish and Sue, 1998).

Preparation of MS basal medium

i. The MS basal medium was made up of 100ml macronutrients, 1ml micronutrients, 1ml iron source, 1ml Potassium iodide and 10ml vitamins added into 2L beaker containing 200ml of distilled water and a magnetic stirrer dropped in it.
ii. Thirty gram (30g) sucrose, 0.1g myo-inositol and 1ml of glycine (0.01g glycine in 5 ml of distilled water) were added and then stirred until dissolved fully. Plant Growth Regulators i.e. Auxin (NAA) and Cytokinin (BAP) were also added first singly and then in combination as per required with one hormone free media as control.
iii. The volume was made up to approximately 1000ml with distilled water in a 1L measuring cylinder then poured back into the 2L beaker. The pH was adjusted to 5.70±0.1 with 1N NaOH or HCl using the pH meter.
iv. Nine grams (9g) of agar was added to the 1000ml prepared MS basal media followed by 0.25g of grinded antibiotic and stirred for complete mixing. Then it was heated in a microwave oven (Hot plate improvised) to dissolve the agar.
v. Approximately 50 ml of the medium was distributed to each sterile media (Bama) bottle. The bottles were labelled based on hormone type and concentration before autoclaving.
vi. The culture medium was autoclaved for 1 hr at 121°C and 15Psi (Villamor, 2010).

Inoculation of explants

Sterilized seeds were inoculated aseptically into the media prepared under the laminar flow hood. The fan of the laminar flow hood was put on and sterilized air was made to flow in the hood sucking up dust in the air around and preventing it from falling into the media which could lead to media contamination. Spirit lamp was lit and the opening of each media bottle was sterilized by passing it over the fire of the lamp to destroy bacteria which could facilitate contamination. The sterilized forceps was used and four seeds were inoculated into each bottle (Patience, 2010).

Incubation

Cultured bottle containing inoculated explants were kept first in the dark for 3days then each cultured bottle was kept in the growth chamber at 25±2ºC under fluorescent light and then monitored for germination and growth.

OBSERVATION AND COLLECTION OF DATA

After incubation, the following parameters were studied and recorded.

Parameters Studied

Germination was considered complete once radicle was visible. The following parameters were studied.

i. Days to explants germination was determined by observing the number of days to the commencement of germination after inoculation of the explants.

ii. Percentage germination (PG) was determined using;

$$PG = \frac{\text{No. of seeds germinated per treanttment}}{\text{No.of seeds innoculated in same treatment}} \times 100$$

(Raun *et. al.,* 2002).

iii. Seedling vigor was determined based on morphological appearance and seedling emergence adopting the procedure of (Gibson, 1980). A scale of 01-5 where

1 = Excellent vigor
2 = Very good
3 = Good
4 = Fair
5 = Very poor vigor.

iv. Number of leaves was determined by counting.

v. Seedling height (SH) and Root length (RL) were determined by spreading a thread against the length of a seedling and root after which it was placed on a ruler to get height and length of seedling and root (Tahir *et. al*., 2013).

DATA ANALYSIS

Data generated from the study was analyzed using Analysis of Variance (ANOVA). To further test for significant difference, the data was subjected to Duncan Multiple Range Test (DMRT) using SAS V9.2 statistical package. Least Significant Difference (LSD) was also used to compare treatment means at ($P < 0.05$) as adopted by (Tahir *et al*., 2011)

RESULTS

Media supplemented with NAA showed rapid growth with thin strong stems and numerous adventitious roots within 29 days of incubation while media supplemented with BAP showed gradual growth with thick stems but no adventitious roots (Plates 1-5).

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Plate 1: Nine (9) weeks old Okra plantlets in a rooting media (0.15mg/L of NAA)

Plate 2: Adventitious rooting in nine (9) weeks old Okra (0.15mg/L of NAA)

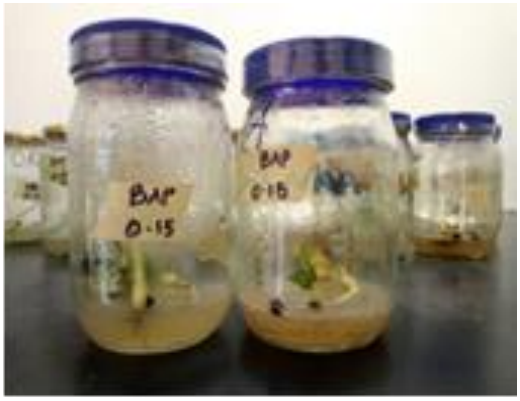

Plate 3: Rooting in nine (9) weeks old Okra (0.15mg/L of BAP)

Media supplemented with combined treatments of BAP and NAA showed gradual growth similar to that seen in media supplemented with single treatment of BAP within 29days (Plates 10, 17).

Plate 4: Nine (9) weeks old Okra plantlets in a combined BAP and NAA media (0.1mg/L *0.1mg/L)

Hormone free media (Control) and media with NAA single treatments showed similar growth appearance, similar nature of stems, roots and leaves (Plates 5, 6).

Plate 5: Nine (9) weeks old Okra in a hormone free media (Control)

Media supplemented with NAA at concentration of 0.25mg/L showed no significant difference in Days to germination, Number of leaves, Percentage germination, Vigor, Seedling height and Root length when compared with the control at $P < 0.05$. Significant differences was seen in Days to germination and Percentage germination in media supplemented with 0.1mg/L NAA, in Days to germination, number of leaves, percentage germination and root length in media supplemented with 0.15mg/L and only in percentage germination in media supplemented with 2.0mg/L NAA when compared with the control at $P < 0.05$(Table 3).

Table 3: The Effects of Varying Concentrations of NAA on the *in vitro* Regeneration of Okra

Conc. (mg/L)	DG (days)	VG	NL	PG (%)	SH (cm)	RL (cm)
Control	0.75±0.49^a	0.25±0.16^a	0.50±0.32^a	6.25±4.09^c	4.59±3.06^a	1.00±0.75^a
0.1	3.00±0^b	0.75±0.61^a	0.75±0.52^a	25.0±0^b	3.09±2.26^a	1.31±0.90^a
0.15	2.88±0.44^b	0.87±0.12^a	2.75±0.49^b	21.9±3.12^{ab}	12.8±2.82^a	6.28±1.65^b
0.2	1.50±0.56^a	0.25±0.16^a	0.50±0.32^a	12.5±4.72^{bc}	2.66±2.28^a	1.73±1.31^a
0.25	1.25±0.54^a	0.50±0.37^a	1.00±0.65^a	9.38±4.57^c	3.39±3.17^a	0.83±0.56^a
P value	**0.0027***	**0.6094**	**0.0107***	**0.0039***	**0.0692**	**0.0068***

Key: DG - Days to germination, **VG** - Vigor, **NL** - Number of leaves, **PG** - Percentage germination, **SH** - Seedling height, **RL** - Root length

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Data were collected after 19days of culture on MS media supplemented with different concentration of NAA; Values are given as mean ± standard error (SE) from two replicated experiments. And those followed by a different letter within a column are significantly different at P < 0.05
However, media supplemented with 0.1mg/L, 0.15mg/L, 0.2mg/L, 0.25mg/L BAP showed no significant difference in days to germination, vigor, number of leaves, percentage germination, seedling height and root length when compared with the control treatment at P < 0.05. The P values of each parameter treated with varying concentrations of BAP were greater than P < 0.05 (Table 4).

Table 4: The effects of varying concentrations of BAP on the *in vitro* propagation of okra

Conc. (mg/L)	DG (days)	VG	NL	PG (%)	SH (cm)	RL (cm)
Control	0.75±0.49ᵃ	0.25±0.16ᵃ	0.50±0.32ᵃ	6.25±4.09ᵃ	4.50±3.06ᵃ	1.00±0.754ᵃ
0.1	1.87±0.54ᵃ	0.63±0.26ᵃ	1.00±0.37ᵃ	15.5±4.57ᵃ	4.50±1.74ᵃ	1.93±0.77ᵃ
0.15	2.62±0.37ᵃ	0.50±0.18ᵃ	1.00±0.37ᵃ	21.9±3.12ᵃ	3.46±1.37ᵃ	1.76±0.67ᵃ
0.2	1.75±0.67ᵃ	0.75±0.49ᵃ	0.50±0.32ᵃ	12.5±4.72ᵃ	0.75±0.49ᵃ	0.64±0.42ᵃ
0.25	1.33±0.54ᵃ	0.25±0.16ᵃ	0.50±0.32ᵃ	9.38±4.57ᵃ	2.26±1.46ᵃ	0.61±0.40ᵃ
P value	**0.1474**	**0.6473**	**0.6529**	**0.1172**	**0.5422**	**0.4234**

Key: DG - Days to germination, **VG** - Vigor, **NL** - Number of leaves, **PG** - Percentage germination, **SH** - Seedling height, **RL** - Root length.
Data were collected after 19days of culture on MS media supplemented with different concentration of NAA; Values are given as mean ± standard error (SE) from two replicated experiments. And those followed by a different letter within a column are significantly different at P < 0.05 according to Duncan Multiple Range Test.

BAP and NAA single treatment on days to germination
The control showed early germination (about 1day) followed by media treated with 0.25mg/L of both NAA and BAP. Media treated with0.1mg/L, 0.15mg/L NAA and media supplemented with 0.1mg/L, 0.15mg/L showed late germination of okra (Fig. I).

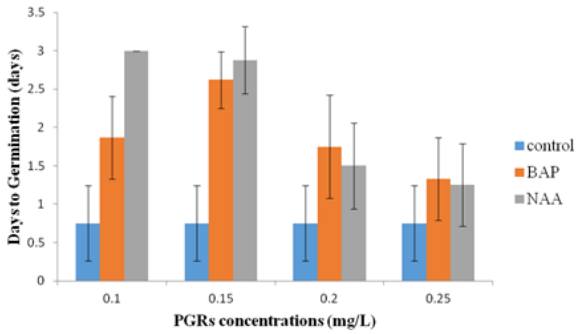

Figure I: Effects of BAP and NAA added singly on days to germination
Key: BAP= 6-BenzylAminoPurine. NAA= 1-NaphthaleneAcetic Acid
PGRs = Plant Growth Regulators

BAP and NAA single treatment on vigor
Best vigor was seen in media treated with 0.15 mg/L NAA. Media supplemented with 0.1mg/L NAA and 0.2mg/L BAP gave a good appearance also followed by media treated with 0.2mg/L and 0.1mg/L BAP (Fig. II).

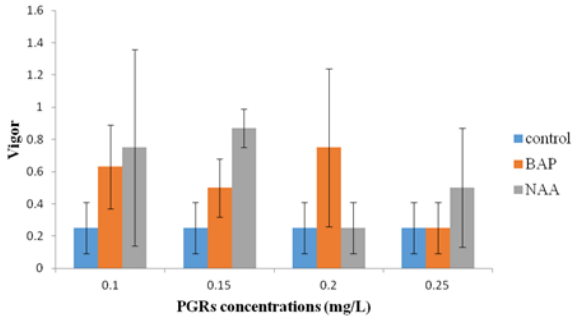

Figure II: Effects of BAP and NAA added singly on vigor
BAP= 6-BenzylAminoPurine. **NAA=** 1-NaphthaleneAcetic Acid**.**
PGRs = Plant Growth Regulators

BAP and NAA single treatment on number of leaves
Media treated with 0.15mg/L NAA gave the highest number of leaves and branches followed by media supplemented with 0.25mg/L NAA, 0.1mg/L and 0.15mg/L BAP (Fig. III).

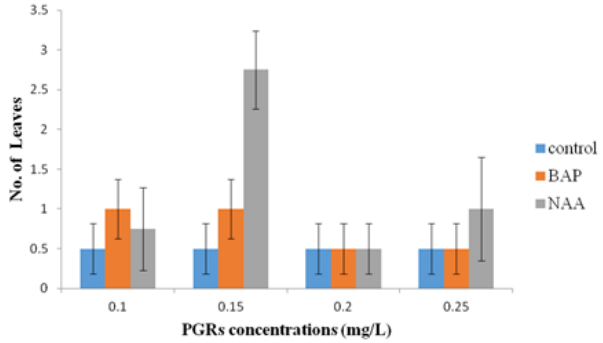

Figure III: Effects of BAP and NAA added singly on number of leaves
BAP= 6-BenzylAminoPurine**. NAA= 1-**NaphthaleneAcetic Acid**.**
PGRs = Plant Growth Regulators

BAP and NAA single treatment on percentage germination
Percentage germination of 25% was seen in media treated with 0.1mg/L NAA followed by media treated with 0.15mg/L BAP and NAA (22%) followed by media treated with 0.1mg/L BAP (15%) (Fig IV).

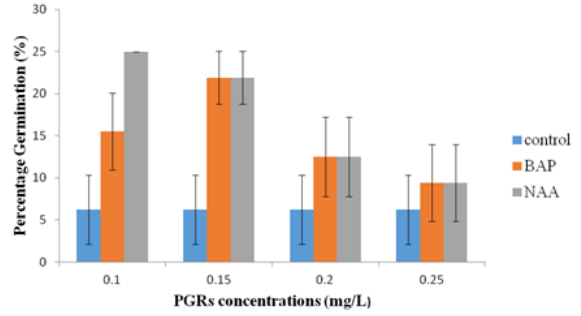

Figure IV: Effects of BAP and NAA added singly on Percentage Germination**.**
BAP= 6-BenzylAminoPurine**. NAA=** 1-NaphthaleneAcetic Acid**.**
PGRs = Plant Growth Regulators

BAP and NAA single treatment on seedling height (SH)
Media supplemented with 0.15mg/L NAA gave better performance (12.8cm). Media treated with 0.10mg/L BAP had same performance with the control media (Fig. V).

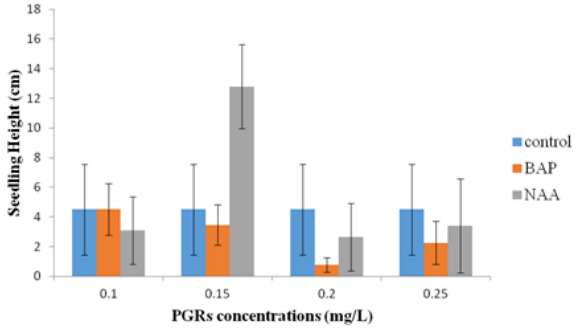

Figure V: Effects of BAP and NAA added singly on seedling height**.**
BAP= 6-BenzylAminoPurine**. NAA=** 1-NaphthaleneAcetic Acid**. PGRs =** Plant Growth Regulators

BAP and NAA single treatment on root length
Media treated with 0.15mg/L NAA gave a better performance in root length (6.5cm) than the control media which are hormone free media (Fig. VI).

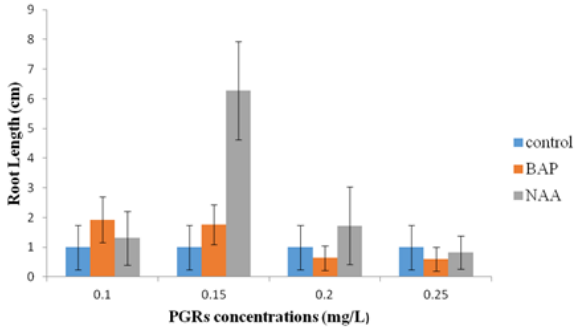

Figure VI: Effects of BAP and NAA added singly on root length.
Key: BAP= 6-BenzylAminoPurine**. NAA=** 1-NaphthaleneAcetic Acid. **PGRs =** Plant Growth Regulators

Media treated with 0.15mg/L + 0.15mg/L (BAP and NAA in combination) showed no significant difference in days to germination, vigor, number of leaves, percentage germination and seedling height when compared with the control treatment at P < 0.05. Media treated with 0.1mg/L + 0.1mg/L showed significant differences in days to germination, number of leaves, percentage germination and root length when compared with the control treatment at P < 0.05. Media supplemented with 0.2mg/L + 0.2mg/L was significantly different days to germination and percentage germination when compared with the control treatment at P < 0.05. Media treated with 0.25mg/L + 0.25mg/L showed significant difference in days to germination, vigor, and number of leaves, percentage germination and root length in comparison with the control treatment at P < 0.05 (Table 5).

Table 5: The Effects of Varying Concentrations of NAA and BAP on the *in vitro* propagation of Okra

Conc. (mg/L)	DG (days)	VG	NL	PG (%)	SH (cm)	RL (cm)
Control	0.75±0.49^{b}	0.25±0.16^{a}	0.50±0.32^{b}	6.25±4.09^{b}	4.59±3.06^{a}	1.00±0.75^{bc}
0.1+ 0.1	3.00±0^{a}	1.88±0.54^{a}	1.75±0.25^{a}	25.0±0^{a}	4.74±1.07^{a}	3.18±0.67^{a}
0.15+ 0.15	1.13±0.54^{b}	0.75±0.49^{a}	0.50±0.32^{b}	9.38±4.57^{b}	0.81±0.56^{a}	0.68±0.55^{c}
0.2 + 0.2	2.88±0.44^{a}	0.88±0.61^{a}	0.75±0.36^{b}	21.9±3.12^{a}	1.91±0.95^{a}	1.01±0.51^{bc}
0.25 + 0.25	3.25±0.16^{a}	1.63±0.56^{a}	2.00±0^{a}	25.0±0^{a}	4.91±1.05^{a}	2.81±0.47^{ab}
P value	**0.0001***	**0.1605**	**0.0006***	**0.0001***	**0.2547**	**0.0134**

Key: DG - Days to germination, **VG** – Vigor, **NL** - Number of leaves, **PG** - Percentage germination, **SH** - Seedling height, **RL** - Root length.

Data were collected after 19days of culture on MS media supplemented with different concentration of NAA; Values are given as mean ± standard error (SE) from two replicated experiments. And those followed by a different letter within a column are significantly different at P < 0.05 according to Duncan Multiple Range Test.

BAP and NAA combined treatment on days to germination (DG)
Early germination was observed in the control media followed by media treated with 0.15 mg/L + 0.15 mg/L BAP and NAA combined treatment (1 day). Late germination was observed in media supplemented with 0.25 mg/L + 0.25 mg/L BAP and NAA combined treatment (Fig. VII).

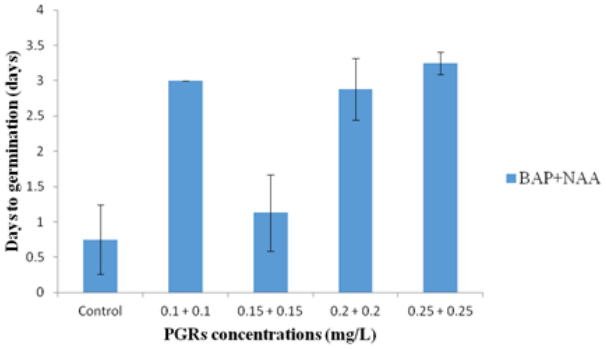

Figure VII: Showing the effects of combined treatment of BAP and NAA on days to germination.
Key: BAP= 6-BenzylAminoPurine**. NAA=** 1-NaphthaleneAcetic Acid**. PGRs =** Plant Growth Regulators

BAP and NAA combined treatment on vigor
Best vigor was seen in media treated with 0.1mg/L + 0.1mg/L BAP and NAA combined treatment followed by media treated with 0.25mg/L + 0.25mg/L combined treatment. Poor vigor was observed in media supplemented with 0.15mg/L + 0.15mg/L BAP and NAA combined treatment followed by 0.20mg/L + 0.20mg/L BAP and NAA combined treatment at P < 0.05 (Fig. VIII).

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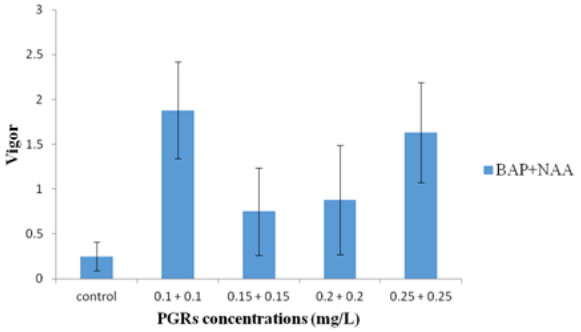

Figure VIII: Effects of combined treatment of BAP and NAA on Vigor**.**
Key: BAP= 6-BenzylAminoPurine**. NAA=** 1-NaphthaleneAcetic Acid**. PGRs =** Plant Growth Regulators

BAP and NAA combined treatment on number of leaves
Highest number of leaves was obtained in media supplemented with 0.25mg/L + 0.25mg/L BAP and NAA combined treatment followed by media supplemented with 0.1mg/L + 0.1mg/L BAP and NAA combined treatment (Fig. IX).

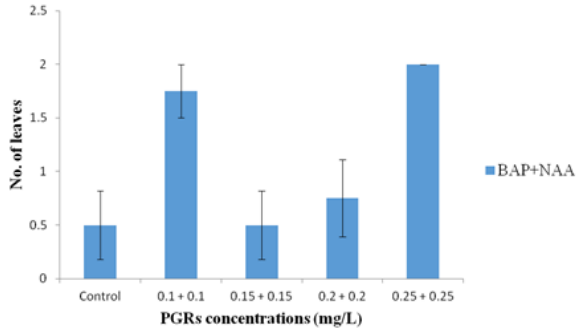

Figure IX: Showing the effects of combined treatment of BAP and NAA on number of leaves/
Key: BAP= 6-BenzylAminoPurine**. NAA=** 1-NaphthaleneAcetic Acid**. PGRs =** Plant Growth Regulators

BAP and NAA combined treatment on percentage germination
Media supplemented with BAP and NAA combined treatment at a concentration of 0.25mg/L + 0.25mg/L, 0.1mg/L + 0.1mg/L showed very good performance. Least performance was observed in media treated with BAP and NAA combined treatment at 0.15mg/L + 0.15mg/L concentration (Fig. X).

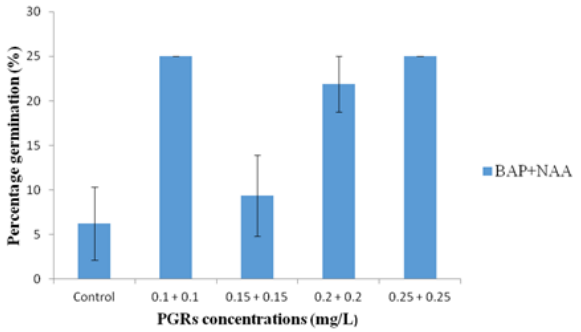

Figure X: Effects of combined treatment of BAP and NAA on percentage germination/

Key: BAP= 6-BenzylAminoPurine**. NAA=** 1-NaphthaleneAcetic Acid**. PGRs =** Plant Growth Regulators

BAP and NAA combined treatment on seedling height
In media supplemented with 0.25mg/L + 0.25mg/L and 0.1mg/L + 0.1mg/L BAP and NAA combined treatment, best performance was obtained at $P < 0.05$ in comparison with the MS control media (Fig. XI).

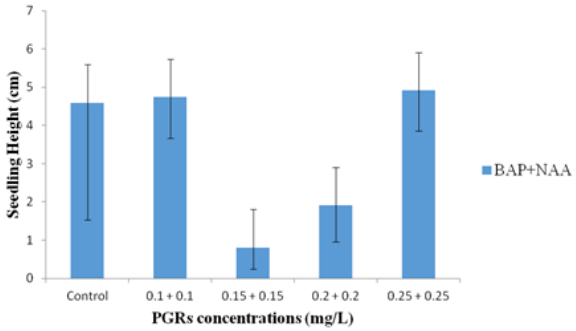

Figure XI: Effects of combined treatment of BAP and NAA on seedling height**.**
Key: BAP= 6-BenzylAminoPurine**. NAA=** 1-NaphthaleneAcetic Acid**. PGRs =** Plant Growth Regulators

BAP and NAA combined treatment on root length (RL)
Media treated with 0.1mg/L + 0.1mg/L BAP and NAA combined treatment performed better followed by media supplemented with 0.25mg/L + 0.25mg/L BAP and NAA combined treatment in comparison with the control at $P < 0.05$ Poor rooting was observed in media treated with 0.15mg/L equal concentrations of BAP and NAA (Fig. XII).

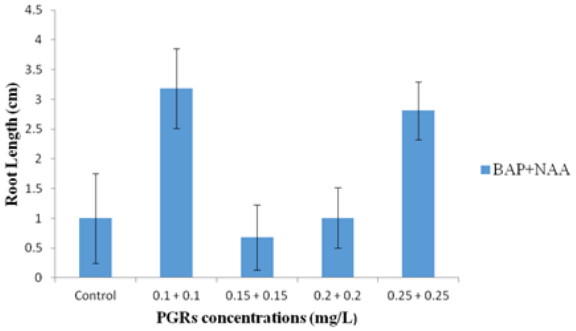

Figure XII: Effects of combined treatment of BAP and NAA on root length**.**
Key: BAP= 6-BenzylAminoPurine**. NAA=** 1-NaphthaleneAcetic Acid**. PGRs =** Plant Growth Regulators

DISCUSSION

Days to germination
Seedling emergence was observed at most 3days after incubation in both single and combined treatments of BAP and NAA in relation to the control. However early germination was observed in control media followed by media treated with 0.25mg/L of both NAA and BAP in single treatments and 0.15mg/L + 0.15mg/L BAP and NAA combined treatments. Similar results were obtained in the work of Umar (2015) and Samaila (2015) on the effects of cytokinins and auxins on the *in vitro* regeneration of *Vigna*

ungulata and *Sorghum bicolor.* They recorded 3days as the highest number of days taken for all treatments including the control also to germinate. Kalimuthu *et al., (*2007) reported that MS medium supplemented with certain concentrations of plant growth regulators influenced seed germination. In the present study, NAA (0.20mg/L and 0.25mg/L) enhanced early germination of the okra seeds in 1.50 ± 0.56^a and 1.25 ± 0.54^a days.

Auxins at a very low concentration promote germination but these effects are subjected to variation depending upon form and species of plant as in this present study; okra. Similarly, the cytokinin (BAP) positively influenced the physiological response of the seed germination as reported by (Thomas, 1989; Syed, 2001). BAP at 0.20mg/L and 0.25mg/L also increased germination in 1.33 ± 0.54^a and 1.75 ± 0.67^a days but is less efficient than that observed in auxin (Someswar and Bikramjit, 2014). All seeds responded to treatments when kept under the light although germination commenced when the treatments were kept in the dark for 3days. Similar result was obtained by Jamaleddine *et al.* (2011) who reported that seeds of *A. annua* germinated after exposure to dark.

Vigor

The best vigor was recorded in media supplemented with 0.15mg/L NAA and 0.1mg/L + 0.1mg/L combined treatments of BAP and NAA followed by 0.25mg/L + 0.25mg/L combined treatment of BAP and NAA, 0.2mg/L of BAP and 0.1mg/L of NAA. This is contrary to the findings of Umar, (2015) who recorded best vigor in the MS control media followed by media treated with combined treatments of 2, 4-D and NAA (Auxins). Cytokinins such as BAP were observed to influence a diversity of responses when applied to plant tissues, or organs. Consequently, it has been used for the induction of organogenesis in several plants (Baskaran and Jayabalan, 2005). The BAP enhanced lateral bud growth by promoting cell division in shoot meristems, influencing the development of vascular tissues, promoting the development of shoots from tissues cultured that are undifferentiated (Graham, *et al*., 2006). It was also reported to influence a multitude of morphological and physiological processes, including cell division and elongation, and the morphological appearance of cultured tissues. (Syed, 2001; Thomas, 1989).

Number of leaves

Maximum number of leaves was recorded in media augmented with 0.15mg/L NAA and 0.25mg/L + 0.25mg/L combined treatments of BAP and NAA followed by 0.1mg/L + 0.1mg/L combined treatments of BAP and NAA. This present study indicates that at a very low concentration, NAA stimulates cell elongation, growth and development responses. Auxin response is related to a concentration, which is normally extremely low thus, the endogenous level of auxin is important in determining the course of development. A high concentration is inhibitory, while a low concentration is stimulatory, and both are important (Syed, 2001). This agrees with the findings of Paulos *et. al.* (2013) who recorded that media supplemented with low concentration of NAA (0.15mg/L) and in a combined treatment of BAP and NAA gave maximum number of leaves in his work on the effects of PGRs on *in vitro* shoot and proliferation of *Musa paradisiaca*.

Percentage germination

Best performance was seen in media supplemented with 0.1mg/L NAA and 0.1mg/L + 0.1mg/L, 0.25mg/L + 0.25mg/L BAP and NAA combined treatment with 25% germination than the rest of the concentrations. Although media treated with NAA alone at a very low concentration also produced a percentage germination of 25% confirms the role of NAA in seed germination. The present study showed that the early germination (3days) and high percentage germination (25%) observed is attributed to the combined effects of equal concentrations of BAP and NAA in stimulating early seed germination. Both phytohormones are important in *in vitro* propagation. Thus, this agrees with the findings of Ezeibekwe *et al.,* (2009) who recorded that the endogenous contents of these two hormones are low in *D. rotundata,* hence their combination complements each other.

Seedling Height

Highest height of seedling was seen in media supplemented with 0.15mg/L NAA single treatment (12.8 cm) followed by 0.25mg/L + 0.25mg/L combined treatments of BAP and NAA (4.74 cm). The control MS media showed similar growth as that seen in media treated with 0.15mg/L NAA (Table 1) although it differs in times of height. This result identifies the role of auxin in stimulating cell elongation, growth and development thus controlling seedling orientation. Plant growth regulators are applied exogenously to produce a variety of growth effects because endogenous hormones are usually at sub optimal levels as low as 1µMin plant tissues (Pessarakli, 2002). This is contrary to the findings of Gonzalez *et al*. (2014) who recorded that the highest growth was observed in hormone free media for *Crytochilum loxense* but is in concord with the work of Samaila (2015) on the effects on NAA, KIN and 2, 4-D on the *in vitro* micro propagation of *Sorghum bicolor,* the work of Ezeibekwe *et al.,* (20009) who recorded that MS media supplemented with a very low concentration of 0.5mg/L NAA in single treatment was optimal for production of higher fresh weight compared to other treatments on the *in vitro* propagation of *Discorea rotundata.*

Root Length

Highest root length was seen in media treated with 0.15mg/L NAA single treatment (6.28 cm) followed by media supplemented with 0.1mg/L + 0.1mg/L BAP and NAA combined treatments (3.18cm) and 0.25mg/L + 0.25mg/L combined BAP and NAA treatments (2.81 cm). The present study showed that a very low concentration of NAA coupled with the presence of an endogenous auxin in the okra plant initiated organogenesis leading to the formation of roots in the okra plant. The result of this study illustrates the importance of auxins in promoting rooting. According to Sandhu *et al.,* (1989), root formation is induced more effectively than by other plant growth regulators including cytokinins. Because auxin moves basipolarly, it was logical to believe that root formation at the basal end is a consequence of the movement of auxin to the lower tissues by gravity (Tamas, 1987).

Auxin as a chemical stimulates cell elongation and influence a wide range of growth and development response. This is contrary to the findings of Bakrim *et al.,* (2007) who recorded that NAA inhibited root elongation for tomato (*Lycopersicum esculentum).*

The combined treatments of BAP and NAA in equal concentrations gave a relatively lesser length of roots which indicates that the effect of one hormone (BAP) inhibits the effect of the other (NAA) in root formation. Both auxin and cytokinin interact in a complex manner to control many aspects of growth

and differentiation. Cato *et al.,* (2013) reported that the two plant hormones act synergistically to regulate cell division, and antagonistically to control lateral bud or root outgrowth. The manipulation of cytokinin levels in plants by exogenous application results in more auxin.

Conclusion

The media augmented with NAA (0.15mg/L) in single treatment had the best performance on the days to germination, vigour, number of leaves percentage germination, seedling height and root length compared to the other treatments and the control It can be used to obtain sterile and uniform starting material for various *in vitro* studies. Further research should be carried out in investigating the effects of other exogenous hormones on the *in vitro* propagation of okra. Similarly, endogenous hormones should be extracted from plants and used to investigate their effects on the *in vitro* propagation of okra and other plants.

REFERENCES

Abdelmageed, A. H. A. (2010). Mode of inheritance of pod spininess in okra (*Abelmoschus esculentus* (L.) Moench. *Tropical and Subtropical Agroecosystems.* 12: 405- 409.

Adams, C. F. (1975). *Nutritive value of American foods in common units, U.S. Department of Agriculture*. Agric Handbook, pp29.

Adetuyi, F. O., Osagie, A. U. and Adekunle, A. T. (2008). Effect of postharvest storage techniques on the nutritional properties of Benin indigenous okra *Abelmoschus esculentus* (L) Moench. *Pakistan Journal of Nutrition* 7: 652-657.

Akogo Y, Aidam A, Odah, K. and Quashie, A. M. (1996). *In vitro* micropropagation of two species of okra (*Abelmoschus esculentus* and *Abelmoschus cannabinus)*. Cahiers Agricultures. 5:109-111.

Al-Ghzawi, A. M., Zaittoun, S. T., Makadmeh, I. and Al-Tawaha, A.R.M. (2003). The impact of wild bee on the pollination of eight okra genotypes under semi-arid Mediterranean conditions. *International of Journal Agricultural Biology,* 5: 409-411.

Arapitsas, P. (2008). Identification and quantification of polyphenolic compounds from okra seeds and skins. *Journal of Food Chemistry*, 110 (4): 1041-1045.

Ariyo, O. J. (1993). Genetic diversity in West African Okra (*Abelmoschus caillei* (A.Chev.) Stevels) Multivariate analysis of morphological and agronomic characteristics. Genetic Resources and Crop Evolution. 40:125-132.

Aykroyd, W. R. (1963). Indian Council of Medicinal Research. Special Report Series. 6 edition. 42:32.

Bajguz, A. and Piotrowska, A. (2009). Conjugates of Auxin And Cytokinin. *Phytochemistry*, 70 (8): 957-969.

Baskaran, P. and Jayabalan, N. (2005) An efficient micropropagation system for *Ecliptaalba* .A valuable medicinal herb.*In vitro Cell* Developmental Biology. *Plant* 41: 532-539.

Bakrim, A., Lamhamdi, M., Sayah, F. and Chibi, F. (2007). Effects of Plant Hormones and 20-hydroxyecdysone on Tomato (*Lycopersicum esculentum*) Seed germination and Seedling growth. *African Journal of Biotechnology Vol. 6(24):2792-2802.*

Baloch A. F. (1994). *Vegetable Crops Horticulture*. National Book Foundation, Islamabad. Pp529-531.

Bish, I. S., Mahajan, R. K. and Rana, R. S. (1995). Genetic diversity in South Asian okra (*Abelmoschus esculentus*) germplasm collection. *Annuals Applied Biology*. 126:539-550.

Cato, S.C., Macedo, W.R., Peres, L.E.P. and Castro, P.R.C. (2013). Sinergism among auxins, gibberellins and cytokinins in tomato cv. Micro-Tom. *Horticultura Brasileira* 31: 549-553.

Chauhan D. V. S. (1972). *Vegetable Production in India*. 3rd Edition, Ram Prasad and Sons, Agra, India. p352.

Chopra, R.N., Nayar, S.L., and Chopra. I.C. (1986). *Glossary of Indian Medicinal Plants*. Council of Scientific and Industrial Research, New Delhi. Pp 150.

Cook, D. A., Bailey, A. and Brown, A. (1991). Somatic embryogenesis in okra. *Plant Physiology*. 1:96-97.

Dhankar, S.K., Holmer, R., Linwattana, G., Nath, P. and Keatinge, J. (2013). *Genetic Improvement of adopted Okra Cultivars for YVMV Disease resistance involving Wild relatives in genus Abelmoschus*. Proceedings Seaveg 2012, Chiang Mai, Thailand, 24-26 January 2012. High Value Vegetables in Southeast Asia: Production, Supply and Demand. Pp 56-60

Düzyaman, E. and Vural, H. (2001). Evaluation of Pod Characteristics and Nutritive value of Okra Genetic resources. Acta Hort ISHS 598: 103-110.

ECHO. (2003). ECHO Technical Note Educational Concerns for Hunger Organization. Rretrieved from www.ecotech.org/technical/technotes. (Accessed on 12th July, 2018).

Ezeibekwe, C.L, Ezenwaka, F.N., Mbagwu and Unamba, C.I.N. (2009). Effects of Combination of Different Levels of Auxin (NAA) and Cytokinin (BAP) on *in vitro* propagation of Discorea rotundata L. (White Yam). *Journal of Molecular Genetics, 1:18-22.*

FAOSTAT. (2008). Food and Agricultural Organisation of the United Nations. On-line and Multilingual Database, http://faostat.fao.org/faostat/

Fajinmi, A. A. and Fajinmi, O. B. (2010). Incidence of okra mosaic virus at different growth stages of okra plants (*Abelmoschus esculentus* (L.) Moench) under tropical condition. *Journal of General and Molecular Virology* 2: 28-31.

Fatimah, B.M. (2003). *An introduction to Biostatistics*. Samarib Publishers, Kano, Nigeria. Pp19-20.

Fishel, F.M. (2006). Institute of Food and Agricultural Sciences. University of Florida. Document No. PI-102 (http://edis.ifas.ufl.edu).

FAO. (1988). *Food and Agricultural Organisation of the United Nations*. Conference on medicinal plants, Rome. pp23.

Ganesan, M., Chandrasekar, R., Kumari, B. and Jayabalan, N. (2007). Somatic Embryogenesis and Plant Regeneration of *Abelmoschus esculentus* through Cuspension culture 51(3): 414-420.

Ghasempour, H. R., Soheilikhah, Z. H., Zebarjadi, A. R., Ghasempour, S. and Karimi, N. (2014). *In vitro* Micropropagation,Ccallus induction and shoot Regeneration in safflower L. cv. Lesaf'. Iranian Journal of Plant Physiology, 4 (2): 999-1004.

Gibson, M.S. (1980). Measurement of Vigor in Seeds or Seedlings. *Sugabeet Research and Extension Reports*, 11,143-147.

Gonzalez, Y. and Cueva, A.A. (2014). Effects of photoperiod, Plant Growth Regulators and Culture media on *in vitro* growth of seedlings of Crytochilum loxense (Lindl.) Kraenzl.

An Endemic and Endangered Orchid from Ecuador. *Revista Peruana de Biologia, 21(2):189-192*.

Graham, L.E., Graham, J.M. and Wilcox, L.W. (2006). *Plant Biology*. Second Edition.Sheri L. Snavely.Pearson Education, Inc.United State of America.Pp 670.

Haider, S.A., Islam, R., Kamal, A.H.M., Rahman, S.M. and Joarder, O.I. (1993). Direct and indirect organogenesis in cultured Hypocotyl explants of *Abelmoschus esculentus* L. Moench. *Plant Tissue Culture*. 3(2): 85-89.

Hamon, S., Charrier A., Koeklin, J. and Van Sloten, D. H. (1990). *Potential contributions to okra breeding through the study of their genetic resources*. In: Report of an International workshop on okra genetic resources. NBPGR. New Delhi, India. 133p.

Jafari, A., Kahrizi, D. and Mansouri, M. (2016). Effects of Plant Growth Regulators and explant on callus induction in pennyroyal (*Mentha pulegium* L.). *Biharean Biologist,* 10 (2): 134-136.

Jamaleddine, Z. O., Lyam, P., Fajimi, O., Giwa, A., Aina, A., Lawyer, E. F., Okere, A. U. and Odofin,W. T. (2011). *In vitro* growth response of *Artemisia annua* seeds to different concentrations of plant growth regulators.*African Journal of Biotechnology*. 10 (77):17841-17844. DOI: 10.5897.

Joshi, A. B ., Gadwal, V. R. and Hardas, M. W. (1974). Okra In Hutchinson, J.B. (ed.), Evolutionary studies in world crops. Diversity and change in the Indian sub-continent, Ed. J B Hutchinson. Cambridge University Press. pp. 99-105.

Kabir A.H., Sarker K.K., Sharmin S. A., Islam M. S., and Alam, M.F.. (2008). Callus induction and Plantlet Regeneration in *Abelmoschus esculentus* (L.) Moench. *Journal of Agricultural Technology* 3(1): 137-142.

Kalimuthu K, Vijayakumar S, Senthilkumar R. (2010). Antimicrobial activity of the biodiesel plant, *Jatropha curcas*. *Intern. J. Pharm. Bio Sci*., 1(3): 1-5.

Kawaguchi,M. Fujioka,S. Sakurai,A. Yamaki,Y.T. and Syono.K. (1993). Presence of a pathway for the biosynthesis of Auxin via indole-3- acetamide in Trifoliata orange. *Plant Cell Physiology*.34:121.

Kirtikar, K.R. and Basu, B.D. (1984). *Indian Medicinal Plants*. 2nd ed., Vol.1, India. Pp293-350.

Kochhar, S. L. (1986). *Tropical Crops.* A textbook of Economic Botany. Macmillan Publishers Ltd. London and Basingstoke, Macmillan Indian Ltd. 467: 263-264.

Koshimizo, K. and Iwamura, H., (1986). *Cytokinins. Chemistry of Plant Hormones*, CRC Press Inc., Florida. USA. 154-199.

Kumar, S., Dagnoko, S., Haougui, A., Ratnadass, A., Pasternak, D. and Kouame, C. (2010). Okra (*Abelmoschus spp*.) in West and Central Africa: Potential and Progress on its Improvement. A special review. *African Journal of Agricultural Research*, 5(25): 3590-3598.

Lamont, W. J. (1999). Okra a versatile vegetable crop. Horticural Technology. 9:179-184.

Liebhardt, W.C. (1976). *Communication in Soil Sciences and Plant Analysis*.Marcel Dekkhor, Inc. pp104-122.

Mitidieri, J. and Vencovsky, R. (1974). Rivista de Agriculture. Brazil. 49:3-6.

Murashige, T. and Skoog, F.(1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Plant Physiology*. 15:473-497.

Murashige, T. (1974). Plant propagation through tissue cultures. *Annual Review Plant Physiology*. 25:135-166.

Nonnecke I. L. (1989). *Vegetable Production*. Van Nostrand Reinhold AVI Publishing. Pp. 608-609.

Norman, J. C. (1992). *Tropical Vegetable Crops*. Arthur, H. Stockwell Ltd., Elms C. Francanbe, Devon. 252pp.

Onunkun, O. (2012). Evaluation of aqueous extracts of five plants in the control of flea beetles on okra (*Abelmoschus esculentus* (L.) Moench. *Journal of Biopesticides*, 5, 62-67.

Oppong-Sekyere, D. (2011). *Assessment of genetic diversity in a collection of Ghanaian Okra germplasm (Abelmoschus spp* L.*) using morphological markers*. MSc. thesis presented to the Department of Crop Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. pp. 42-60.

Pakseresht, G., Kahrizi, D., Mansouri, M., Ghorbani, T. and Kazemi, N. (2016). Study of callus induction and cell culture to secondary metabolite production in *Hyssopus officinalis* L. *Journal of Reports in Pharmaceutical Sciences*, 5 (2): 104-111.

Paulos, M.M., Joshi, V.R. and Pawar, S.V. (2013). Effect of BAP and NAA on *in vitro* Shoot Establishment and Proliferation of Banana (*Musa paradisiaca*) Cv. Grand niane. *International Journal of Science and Research,* ISSN (online):2319-7064.

Pessarakli, M. (2002). Handbook of plant and crop stress. CRC Press, Boca Raton, F.L New York, USA. pp.385–394.

Purseglove, J.W. (1968). *Tropical Crops Dicotyledons*. Longman Group Limited. 368-70.

Purseglove, J. W. (1987). *Tropical Crops. Dicotyledons*. Longman Singapore Publishers Ltd. pp 324.

Rahman, M .T., Hossain, M. J. and Khalekuzzaman, M. (2008**).** *In Vitro* Indirect Plantlet Regeneration from Hypocotyl Segmentsand Cotyledonary Explant derived Calli in Lady's finger (*Abelmoschus esculentus* L. Monech). **J.** bio-sci. 16: 49-57, 2008 ISSN 1023-8654. Retrieved from http://www.banglajol.info/index.php/JBS/index

Ramu, P. M. (1976). Breeding Investigation in Okra (*Abelmoshus esculentus* (L.) Moench).*Mysore Journal of Agricultural Science10* (1):146.

Raun , S., Xue, Q. and Thlkowska, K. (2002). Effect of seed Priming on Germination and Health of Rice (Oryza sativa L.) seeds. *Seed Science and Technology*, 34(1):63-69.

Rehman, H., Ahmed, M. S., & Laghari, M. H. (2003). Effect of culture media and Growth regulators on micro propagation of peach rootstock GF 677. *Pak. J. Bot.*, 35(3), 331-338.

Saifullah, M. and Rabbani, M. G. (2009). Evaluation and characterization of okra (*Abelmoschus esculentus* L. Moench.) genotypes. *SAARC Journal of Agriculture* 7: 92-99.

Salunke, S.B., Qudrathullah, M.D., Bhavsar, C.M. and Deogadkar, B.B. (1988). *Physical and Agrochemistry* (2nd Edition). NiraliPrakashan, Pune. Pp167-190.

Samaila, Z. (2015). *Effects of Naphthaleneamino acid, Kinetin and 2,4-Dichlorophenoxy acetic acid on the in vitro micro propagation of (Sorghum bicolor)*. Pp22-27.

Sandhu, A.S., Singh, S.N., Minhas, P.P.S and Grewal, G.P.S. (1989). Rhizogenesis of Shoot cuttings of raspberry (*Physalis peruviana* L.). *Indian Journal of Horticulture*, 46:376-378.

Somswar, R. and Bikramjit, B. (2014). *In vitro* Micropropagation of *Dendrobium chrysanthum* Wall. ex Lindl. - A threatened Orchid. *Scholars Academic Journal of Biosciences*, 2(1):43-47.

Savello, P. A., Martins, F. and Hull, W. (1980). Nutrition composition of okra seed meals. *Journal of Agricultural Food and Chemistry*. 28:1163-1166.

Shabana, I., Farhat, A.K., Shazia, E., Faisal, N., Aish, M. and Saima, K. (2017). *In vitro* propagation of selected Medicinal plants species. Asian *Journal of Agriculture and Rural Development*, 7(2), 40-47.

Siemonsma, J. S. (1982). *West African okra morphological and cytogenetical indications for the existence of a natural amphidiploid of Abelmoschus esculentus* (L.).PROTA Foundation, the Netherlands, pp.76-89.

Siemonsma, J. S. (1991). West African okra morphological and cytogenetical indicators for the existence of a natural amphidiploid of *Abelmoschus esculentus* (L.) and *Abelmoschus manihot* (L.) Mediks. Euphytica 31:241-252.

Singh, B.P. (1990). Effect of spacing Okra. *Indian J. Agron*. 35(4):439.

Soorni, J. and Kahrizi, D. (2015). Effect of genotype, explant type and 2,4-D on cell dedifferentiation and callus induction in cumin (*Cuminum cyminum* L.) medicinal plant. *Journal of Applied Biotechnology Reports*, 2 (3): 265-270.

Stevels, J. M. C. (1988).Une nouvelle combinaison dans Abelmoschus Medik, un gombo d'Afrique de l'Ouest et Centrale. Bull. Mus. Hist. Naturl, Paris, 4Q série, 10, section B., Adansonia, NO2 137-144. Les apports potentiels, 67:50-52.

Syed, S. M. N. (2001).*Plant Growth Hormones: Growth Promotors and Inhibitors.*Nuclear Institute of Agriculture, Tando Jam, Pakistan. Pp40.

Tahir, S.M., Victor, K., Abdulkadir, S. (2011). Effect of 2,4-D concentration on callus induction in sugarcane. *Nigerian journal of Basic and Applied Sciences.* 19(2):213-217.

Tahir, S.M., Usman, I.S., Katung, M.D. and Ishiyaku, M. (2013). Micro propagation of wormwood (Artemisia annua L.) using leaf Primordia. *Science world Journal.* 8(1):1-2.

Tahir, S.M., Usman, I.S., Katung, M.D. and Ishiyaku, M. (2013). *In vitro* regeneration of *Artemisia annua* (warm wood) using seed explants. *The International Journal of Biotechnology*, 2(11):171-181.

Taimori, N., Abdossi, V. and Papzan, A. H. (2016). Cell dedifferentiation, Callus induction and Somatic embryogenesis in *Crataegus spp*. Cellular and Molecular Biology, 62 (11):100-107.

Tamas, I. A., (1987). In: P.J., Davies, (Ed). *Plant Hormones and Their Role in Plant Growth and Development.*Boston: Martinus Nijhoff.Pp 393.

Tindall, H. D. (1983). *Vegetables in the tropics. Macmillan* Press Ltd. London and Basingstoke. Pp. 25-328.

Thomas, T.H., (1989).Plant Growth Hormones. *Journal of Plant Growth Reguators.l* 8:255

Thompson, H. C. and Kelley, W. C,(1957). *Vegetables Crops*, 5 Edition, McGraw Hill, New York. Pp. 561-563.

Thorpe, T. A. (2007). History of plant tissue culture. *Mol. Biotechnol*. 37, 169–180.

Umar, A. (2015). *Effects of Cytokinin and Auxin on the in vitro regeneration of vigna ugulata* (L.) walp. Pp20-24.

Vaissière, B. E. and Vinson, B. (1994). Pollen morphology and its effect on pollen collection by honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), with special reference to upland cotton, *Gossypium hirsutum* L. (Malvaceae). Grana, 33:128-138.

Valeriana, P. J. (2011). *Training Resource Text on Crop Development, Major Economic Practices, Disease and Insect Ecology, Insect Pests, Natural Enemies and Diseases of Okra*. McGraw Hill, New York. Pp. 40-80

Vasil, I. K., and Vasil, V. (1972). Totipotency and Embryogenesis in plant cell and tissue cultures. *In vitro* 8, 117–127.

Vasil, V., and Vasil, I. K. (1974). Regeneration of tobacco and petunia plants from protoplasts and culture of corn protoplasts. *In vitro* 10, 83–96.

Wiese, A.M and Binning, L.K. (1987). Calculating the threshold temperature of development for Weeds. *Weed Science*, 35, 177-179.

Zinhari, Z., Pourseyedi, S.H. and Zolala, J. (2016). Callus induction and direct shoot regenertion in *Lepidium draba* L. Explants. *Journal of Agricultural Biotechnology*, 8 (2):31-51