EFFECTS OF VARYING CONCENTRATIONS OF PLANT GROWTH
REGULATORS ON THE IN VITRO PROPAGATION OF
AMARANTHUS (AMARANTHUS TRICOLOR L.)

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ABSTRACT
Amaranthus is an important vegetable crop that belongs to the family Amaranthaceae. An experiment was carried out to study the effects of varying concentrations of Plant Growth Regulators (PGRs) on the in vitro propagation of amaranthus seed using seed explants. The seeds were cultured in vitro on Murashige and Skoog (MS) basal media containing different concentrations (0.1mg/L to 0.2mg/L) of auxin (NAA) gibberellin (GA3) and cytokinin (BAP). Early germination was observed in media treated with NAA 0.02mg/L. Best vigor was recorded in media treated with 0.20mg/L BAP treatment. All media has same number of leaves with the exception of 0.2mg/LNAA treatment. Highest root length was observed in media treated with 0.15 mg/L GA3 and 0.2mg/l GA3 and NAA. Highest shoot length was recorded in media treated with 0.15 mg/L GA3. Results of Analysis of Variance (ANOVA) indicated significant differences among the treatments compared with the control (p < 0.05). The protocol developed in this study is suitable for large scale seedling formation, biomass production and obtaining uniform materials for various in vitro studies for the improvement of amaranthus.

Keywords: Growth regulators, Concentrations, In vitro, Amaranthus.

INTRODUCTION
Amaranthus (Amaranthus tricolor), collectively known as amaranth, is a cosmopolitan genus of annual or short-lived perennial plants. Some amaranth species are cultivated as leaf vegetables, pseudo cereals, and ornamental plants. Most of the Amaranthus species are summer annual weeds and are commonly referred to as pigweed (Curtis et al., 2017).

Amaranthus is a catkin-like cymes of densely packed flowers grow in summer or autumn (RHS, 2008). Approximately 60 species are recognized, with inflorescences and foliage ranging from purple, through red and green to gold. Members of this genus share many characteristics and uses with members of the closely related genus Celosia. Amaranthus shows a wide variety of morphological diversity among and even within certain species. Although the family amaranthaceae is distinctive, the genus has few distinguishing characters among the 70 species included (Juan; et al., 2007). This complicates taxonomy and Amaranthus has generally been considered among systematics as a “difficult” genus. Formerly, classified the genus into two subgenera, differentiating only between monocious and dioecious species. Although this classification was widely accepted, further infrageneric classification was and still is needed to differentiate this widely diverse group. Known to the Aztecs as huauhtli, amaranth is thought to have represented up to 80% of their energy consumption before the Spanish conquest (He et al., 2002).

Another important use of amaranth throughout Mesoamerica was in ritual drinks and foods. It is made up about 5% of the total faty acids of amaranth is extracted as a vegetable-based alternative to the more expensive shark oil for use in dietary supplements and cosmetics. The flowers of the amaranth were used by the Hopi (a tribe in the western United States) as the source of a deep red dye. A hypochondriacs flowering, the genus also contains several well-known ornamental plants, such as Amaranthus caudatus (He et al., 2002).

Many members of the Amaranthus family have been rather recalcitrant or dormancy due to poor seed germination, endogenous fungal and bacterial contamination and low viability of the seeds (He et al., 2002). The ability to regenerate Amaranthus plants in vitro would allow the rapid propagation of this important horticultural crop. Processes of plant growth require the action and cross talk of phytohormones such as auxins, cytokinins and gibberellin. By setting phytohormones concentration in the medium, in differences amount, rate and growth patterns of explants can be observed with full accuracy. Plant tissue culture techniques have been widely used for micro propagation of plants of different kind since the early demonstration of cellular totipotency and differentiation in vitro (Murashige, 1974; Tahir et al., 2015).

The whole idea of plant tissue culture is that many plant mature cells are not terminally differentiated but rather retain developmental plasticity within a time, except for certain types of terminally differentiated cells e.g., trachaeate cells, sieve tube cells, and highly lignified cells such as mature fibres and sclereids. Plant cells are capable under certain conditions to differentiate, re-enter the cell cycle, proliferate and regenerate tissues, organs and entire fertile plants. Given the importance of in vitro plant regeneration for a wide range of applications including basic research, micro-propagation, germplasm conservation, and formation of genetically modified plants (Vasil, 1972; Thorpe, 2007).

Plant growth regulators (PGRs) usually are defined as organic compounds, other than nutrients, that in small concentrations, affect the physiological processes of plants. In practical purpose, they are defined as either natural or synthetic compounds that are able to regenerate Amaranthus in vitro, would allow the rapid propagation of this important horticultural crop. Processes of plant growth require the action and cross talk of phytohormones such as auxins, cytokinins and gibberellin. By setting phytohormones concentration in the medium, in differences amount, rate and growth patterns of explants can be observed with full accuracy. Plant tissue culture techniques have been widely used for micro propagation of plants of different kind since the early demonstration of cellular totipotency and differentiation in vitro for use in dietary supplements and cosmetics (Vasil, 1972; Thorpe, 2007).

Plant growth regulators (PGRs) usually are defined as organic compounds, other than nutrients, that in small concentrations, affect the physiological processes of plants. In practical purpose, they are defined as either natural or synthetic compounds that are applied directly to plant to alter its life processes/structure in some beneficial way so as to enhance yield, improve quality and facilitate harvesting (Salunke et al., 1988).
This research work studied the effects of varying concentrations of plant growth regulators necessary to maximize and sustain continuous seed germination as well as high frequency regeneration of shoots of in vitro propagated *Amaranthus tricolor*.

**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°38’E, altitude 670m above mean sea level, 640km from the Atlantic shores of Nigeria in the north, and then transported to the Post Graduate (PG) Laboratory of the Department of Biological Sciences, Kaduna State University (KASU) located on Latitude 10°52’N and 7°44'E Longitude, 614m elevation above sea level, Tafawa Balewa way, Kaduna metropolis, Kaduna, Nigeria.

**Source of Explants**
Seeds of amaranth (*Amaranthus spinosus* L.) were locally collected from Kaduna central market, Kaduna State within the Northern Guinea savannah Nigeria, and was identified by a Plant Taxonomist in the Herbarium unit of the Department of Biological Sciences, Faculty of Science, Kaduna State University. The following voucher number was allocated V/No. KASU/BSH/397.

**Treatment of the explants**
Seeds were subjected to the following treatments;

**Sterilization of explants**

i. Seeds were washed outside the laminar flow hood with household detergent and rinsed with running tap water.

ii. Seeds were washed again with detergent and rinsed with a sterilized distilled water in laminar flow hood.

iii. Seeds were poured again into 70% ethanol for 3 minutes and rinsed with sterilized distilled water.

iv. Seeds were then soaked in 30% mercury chloride for 5 minutes. After this treatment, the seeds were rinsed thoroughly in sterile distilled water to make free the seeds from mercury chloride.

v. Sterilized seeds were then subjected to aseptic conditions so as to germinate in bama bottles (media) containing 50mL full strength MS (Murashige and Skoog, 1962) basal media (Hamish and Sue, 1998).

**Preparation of MS basal medium**
A quantity of 1L of MS basal medium, the required volume of each stock solution 100mL macronutrients and 1mL micronutrients, 1mLuron source, 1mLPotassium iodide and 10mL vitamins were added into 2L beaker containing 200mL of distilled water and a magnetic stirrer dropped in it. Also, 30g sucrose, 0.1g Myo-inositol and 1mLof glycine (0.01g glycine in 5mL 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.

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 using a pH meter.

Similarly, 9g of agar and 0.25g Augmentin antibiotic were added, well stirred and heated in a microwave oven (Hot plate improvised) to dissolve.

Approximately 50 mL of the medium was dispensed into each sterile media bottle. The bottles were labelled based on hormone type and concentration before autoclaving.

The culture medium was autoclaved for 1hr at 121°C temperature and 15Psi before inoculation of the explants (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 so that sterilized air will be 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 flame of the lamp to destroy bacteria which could facilitate contamination. A sterilized forceps was used to inoculate fifty to sixty (50-60) seeds into each bottle.

**Incubation of the Explants**
Cultured bottle containing inoculated explants was kept first in the dark for 36hrs before placing each cultured bottle on the growth chamber at 25±2°C under fluorescent light and then monitored for germination and growth.

**Acclimatization**
Well grown and rooted bud was excised aseptically and the roots were washed thoroughly with running tap to make it free from the MS basal media attached to them. The seedlings were then transferred into soil which consists of top soil and cow dung in the ratio 2:1 in polythene bags. They were then placed in a bigger transparent polythene bag, water was then sprinkled inside followed by blowing air into the bag. The bag was tied and hanged on the rafter of the outside room (Tahir et al., 2015).

**Observation and Collection of Data**
Fourteen days after incubation, the following parameters was studied and recorded.

**Parameters Studied**
Germination of seeds was considered complete once radicle is visible. The following parameters were studied.

Days to explants germination was determined by observing the number of days to the commencement of germination after inoculation of the explants (seeds) once radial is visible. Shooting vigour was determined based on morphological appearance and seedlings emergence adopting the procedure of (Gibson, 1980). A scale of 1-5 will be used where 1 = Excellent vigour. 2 = Very good. 3 = Good. 4 = Fair. 5 = Very poor.
Effects of Varying Concentrations of Plant Growth Regulators on the In Vitro Propagation of Amaranthus (Amaranthus Tricolor L.)

Effects of Varying Concentrations of BAP on In Vitro Regeneration of Amaranthus

Table 1. Effects of Varying Concentrations of BAP on In Vitro Regeneration of Amaranthus

<table>
<thead>
<tr>
<th>CONC</th>
<th>DG</th>
<th>VG</th>
<th>NL(n)</th>
<th>RL(CM)</th>
<th>SL(CM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.50±0.07*</td>
<td>1.00±0.57*</td>
<td>1.00±0.45*</td>
<td>0.60±0.00*</td>
<td>1.58±0.71*</td>
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<tr>
<td>0.10</td>
<td>3.00±0.00*</td>
<td>2.00±0.00*</td>
<td>2.00±0.00*</td>
<td>0.82±0.07*</td>
<td>3.80±0.60*</td>
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<tr>
<td>0.15</td>
<td>2.67±0.20*</td>
<td>1.00±0.00*</td>
<td>2.00±0.00*</td>
<td>2.60±0.30*</td>
<td>4.30±0.30*</td>
</tr>
<tr>
<td>0.20</td>
<td>1.00±0.00*</td>
<td>1.00±0.00*</td>
<td>2.00±0.00*</td>
<td>0.80±0.00*</td>
<td>1.80±0.10*</td>
</tr>
</tbody>
</table>

P-value 0.0001* 0.0027* 0.0005* <0.0001* 0.0015*

DG - days to germination, VG - vigor, NL - number of leaves, RL - root length, SH - seedling height

The control and NAA 0.20mg/L showed early germination (about 1 day) followed by media treated with GA3 0.20mg/L and 0.15mg/L BAP. Media treated with 0.10mg/L BAP, 0.10mg/L GA3, and 0.10mg/L NAA, 0.15mg/L NAA, 0.20mg/L BAP followed by media supplemented with 0.15 mg/L GA3 showed late germination of Amaranthus (Fig. 1).

Plate 1: A 14 days old Amaranthus in GA3 0.15mg/L

Table 2. Effects of Varying Concentrations of GA3 on In Vitro Regeneration of Amaranthus

<table>
<thead>
<tr>
<th>CONC</th>
<th>DG</th>
<th>VG</th>
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<th>RL(CM)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.50±0.67*</td>
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<td>1.00±0.45*</td>
<td>0.60±0.00*</td>
<td>1.58±0.71*</td>
</tr>
<tr>
<td>0.10</td>
<td>3.00±0.00*</td>
<td>2.00±0.00*</td>
<td>2.00±0.00*</td>
<td>0.67±0.10*</td>
<td>3.70±0.10*</td>
</tr>
<tr>
<td>0.15</td>
<td>3.00±0.00*</td>
<td>2.50±0.20*</td>
<td>2.00±0.00*</td>
<td>1.90±0.40*</td>
<td>3.70±0.30*</td>
</tr>
<tr>
<td>0.20</td>
<td>1.50±0.70*</td>
<td>1.00±0.00*</td>
<td>1.00±0.00*</td>
<td>0.00±0.00*</td>
<td>1.00±0.70*</td>
</tr>
</tbody>
</table>

P-value 0.0164* 0.0008* 0.0005* <0.0001* 0.0062*

DG - days to germination, VG - vigor, NL - number of leaves, RL - root length, SH - seedling height

Table 3. Effects of Varying Concentrations of NAA on In Vitro Regeneration of Amaranthus

<table>
<thead>
<tr>
<th>CONC</th>
<th>DG</th>
<th>VG</th>
<th>NL(CM)</th>
<th>RL(CM)</th>
<th>SL(CM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.50±0.67*</td>
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<td>1.00±0.45*</td>
<td>0.60±0.00*</td>
<td>1.58±0.71*</td>
</tr>
<tr>
<td>0.10</td>
<td>3.00±0.00*</td>
<td>2.00±0.00*</td>
<td>2.00±0.00*</td>
<td>0.90±0.00*</td>
<td>3.00±0.10*</td>
</tr>
<tr>
<td>0.15</td>
<td>3.00±0.00*</td>
<td>2.00±0.00*</td>
<td>2.00±0.00*</td>
<td>1.00±0.00*</td>
<td>2.00±0.10*</td>
</tr>
<tr>
<td>0.20</td>
<td>1.00±0.00*</td>
<td>1.00±0.00*</td>
<td>1.00±0.00*</td>
<td>0.00±0.00*</td>
<td>1.00±0.70*</td>
</tr>
</tbody>
</table>

P-value 0.0164* 0.0008* 0.0005* <0.0001* 0.0062*

DG - days to germination, VG - vigor, NL - number of leaves, RL - root length, SH - seedling height
Best vigor was observed in media treated with 0.20 mg/L BAP, followed by 0.15 mg/L NAA. Control and media supplemented with 0.15 mg/L GA3, 0.20 mg/L GA3 and 0.20 mg/L NAA showed least vigor followed by media treated with 0.1 BAP, 0.15 BAP and 0.15 NAA (Fig. ii).

![Fig. ii: Effects of BAP, GA3 and NAA on Vigor](image)

Media treated with all the varying PGRs concentrations gave same number of leaves with the exception of control and 0.20 mg/L NAA. (Fig. iii)

![Fig. iii: Effects of BAP, GA3 and NAA on Number of Leaves](image)

Media treated with 0.15 mg/L GA3 gave a better performance in root length (about 2.5 cm) followed by media treated with 0.1 mg/L BAP and then media treated with 0.15 mg/L BAP, 0.15 mg/L NAA. Control and media treated with 0.1 mg/L NAA showed least root length, followed by 0.2 mg/L BAP and 0.20 mg/L GA3 (Fig. iv).

![Fig. iv: Effects of BAP, GA3 and NAA on Root Length](image)

Media treated with 0.15 mg/L GA3 gave better performance on seedling height (4.3 cm) followed by media treated with 0.10 mg/L BAP, 0.1 mg/L NAA and 0.15 NAA. Control and media treated with 0.20 mg/L BAP, 0.20 mg/L NAA showed the least performance on seedling length followed by 0.1 mg/L BAP, 0.15 mg/L BAP and 0.20 mg/L GA3 media. (Fig. v)

![Fig. v: Effects of BAP, GA3 and NAA on Seedling Height](image)

DISCUSSION

The early germination recorded in media treated with NAA 0.02 mg/L is contrary to the results obtained by Tahir et al., (2019) who studied the effects of varying concentrations of plant growth regulators (PGRs) on the in vitro propagation of Okra where they recorded early germination in the control. Similar result was also recorded by Umar, (2015) where they studied the effect of Cytokinins and Auxins on the in vitro regeneration of Vigna unguiculata and Sorghum bicolor. Similarly, MS media treated with certain concentrations of PGRs influenced early germination. Auxins at a very low concentration were reported to promote germination but the effects depends on the species of the plant (Tahir et al., 2019). Similarly, Thomas, (1989) was of the view that, BAP positively influenced the physiological response of the seed to germination factors. Likewise, BAP at 0.2 and 0.25 concentrations were reported to also promote early germination at 1.33 ± 0.54 days. (Someswar and Bikranjit, 2014). In the present study, response of...
treated seeds to germination after 3 days when exposed to light from the dark agreed with the findings of Jamaleddine (2011). The best vigor recorded in media treated with 0.20mg/L BAP is an indication of the dual functions of BAP in enhancing lateral bud growth by promoting cell division in shoot meristems, influencing the development of Vascular tissues, promoting the development of shoots from undifferentiated tissues of cultured tissues (Taylor et al., 1997; Graham et al., 2006). It also agreed with the findings of Tahir et al., (2019) who recorded best vigor in media of combined treatment of BAP and NAA. However, it is also contrary to the findings of Umar (2015) who recorded best vigor in free media. It had been used for the induction of organogenesis in many varieties of plant (Baskaran and Jayabalal, 2005).

Maximum number of leaves where recorded in all media with the exception of 0.2mg/LNAA in single treatments. Similar result was also obtained by Paulos et al., (2013) who recorded that media treated with low concentration of 0.15mg/L give maximum number of leaves, in his work effects of PGRs on in vitro shoot and proliferation of paradigm. At high concentration are inhibitory while low concentration is stimulatory (Syed, 2001). The highest shoot length recorded in media treated with 0.15mg/L GA3 0.10mg/L GA3 may be attributed to the effect of GA3 in stimulating early growth and development of plants by promoting cell division in epical meristem and cambium tissues. (Taylor, et al., 1997; Tahir et al., 2014). Likewise, GA3 has been used in the shoot proliferation media to improve shoot elongation, rate of multiplication, growth and quality of shoots (Brand and Lineberger 1992).

The highest root length observed with 0.15mg/L GA3 followed by 0.10mg/L BAP treatments is contrary to the findings reported by Tahir et al., 2014 who recorded highest root length in media with 0.15mg/L NAA. It also disagreed with the findings of Amali (2017) studying the direct regeneration potential of Sorghum bicolor under the influence of plant growth regulators in which he recorded best root formation in 1.0mg/L of IBA. Similarly, best root formation was recorded using 0.5mg/L IBA who worked on the effect of plant growth regulators on in vitro propagation of Spinachia oleraceali. A healthy rooting system is vital for tissue culture propagation system for Eclipta alba. A valuable medicinal herb. In vitro Cell Developmental Biology. Plant 41: 532-539.

The findings of Umar (2015) who recorded best vigor in free media. It had been used for the induction of organogenesis in many varieties of plant (Baskaran and Jayabalal, 2005).

Generally, Auxins are known to promote root formation by inducing root more effectively than other plant growth regulators including Cytokinins (Sandhu et al., 1989). Auxin also stimulates cell elongation and influence a wide range of growth and development. It was reported to move basip tally. This is 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). However, both auxin and cytokinin interact in a complex manner to control many aspects of growth and differentiation. It was also reported that the two plant hormones act synergistically to regulate cell division and antagonistically to control lateral bud or root outgrowth Cato et al., (2013).

Conclusion
A significant difference was observed among the treatments compared with the control. A concentration of 0.02mg/L NAA was found to be suitable for rapid germination of the Amaranthus seeds. Best vigor was recorded with 0.20mg/L BAP concentration. Highest root length was observed in media treated with 0.15mg/L GA3 and 0.2mg/L GA3 and NAA. However, 0.15mg/L GA3 produced the highest shoot length. The protocol developed in this study is suitable for large scale in vitro seedling formation and biomass production of amaranthus.

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We wish to acknowledge the untiring support of Dr Maimuna Abdulmalik, the Head of Biotechnology Unit, Dr Alasan Usman, Head of Plant Science Department/ Institute for Agricultural Research (IAR) for providing us with the bench space in their Biotechnology Laboratory to conduct the experiment. We also appreciate the technical support of Mal. Ja’afar Muhammad.

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