

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

*Tahir S.M. and Mathew J.Y.

Department of Biological Sciences, Kaduna State University, Kaduna, Nigeria

*Corresponding Author Email Address: smtahir@kasu.edu.ng

+2347032852090

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 monoecious 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 fatty 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., tracheary elements, 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 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 was 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 amaranthus (*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 3minutes 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, 1mL Iron source, 1mL Potassium 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 1mL of 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 radical 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 vigour

Number of leaves was determined by counting.

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

Experimental design

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

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 GA₃ and NAA showed rapid growth and numerous adventitious roots within 14 days of incubation while media supplemented with GA₃ and BAP combined showed gradual growth.



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

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

CONC.	DG	VG	NL(n)	RL(CM)	SL(CM)
Control	1.50±0.67 ^a	1.00±0.57 ^a	1.00±0.45 ^a	0.60±0.00 ^a	1.58±0.71 ^a
0.10	3.00±0.00 ^{ab}	2.33±0.33 ^b	2.00±0.00 ^b	1.18±0.57 ^{ab}	1.93±0.042 ^a
0.15	2.33±0.40 ^b	2.67±0.22 ^b	2.00±0.00 ^b	0.99±0.03 ^b	1.90±0.18 ^a
0.20	3.00±0.00 ^b	4.33±0.49 ^c	2.00±0.00 ^b	0.68±0.065 ^b	1.58±0.71 ^a
P=value	0.0592	0.0002*	0.0095*	0.0446*	0.9064

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

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. * shows values less than P < 0.05.

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

CON.	DG	VG	NL(n)	RL(CM)	SL(CM)
Control	1.50±0.67 ^a	1.00±0.57 ^a	1.00±0.45 ^a	0.60±0.00 ^a	1.58±0.71 ^a
0.10	3.00±0.00 ^{ab}	2.00±0.00 ^b	2.00±0.00 ^b	0.82±0.07 ^a	3.80±0.60 ^a
0.15	2.67±0.20 ^b	1.00±0.00 ^b	2.00±0.00 ^b	2.60±0.30 ^a	4.30±0.30 ^b
0.20	1.00±0.00 ^b	1.00±0.00 ^b	2.00±0.00 ^c	0.80±1.00 ^b	1.80±0.10 ^b
P value	0.0301*	0.0275*	0.0095*	<0.0001*	0.0015*

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

Concentration	DG	VG	NL(CM)	RL(CM)	SL(CM)
Control	1.50±0.67 ^a	1.00±0.57 ^a	1.00±0.45 ^a	0.60±0.00 ^a	1.58±0.71 ^a
0.10	3.30±0.00 ^{ab}	3.00±0.00 ^b	2.00±0.00 ^b	0.67±0.10 ^b	3.70±0.10 ^{ab}
0.15	3.00±0.00 ^b	2.50±0.20 ^b	2.00±0.00 ^b	1.00±0.40 ^b	3.70±0.30 ^{bc}
0.20	1.50±0.70 ^b	1.00±0.50 ^b	1.00±0.00 ^b	0.00±0.00 ^c	1.60±0.70 ^c
P value	0.0154*	0.0008*	0.00095*	<0.0001*	0.0062*

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. i).

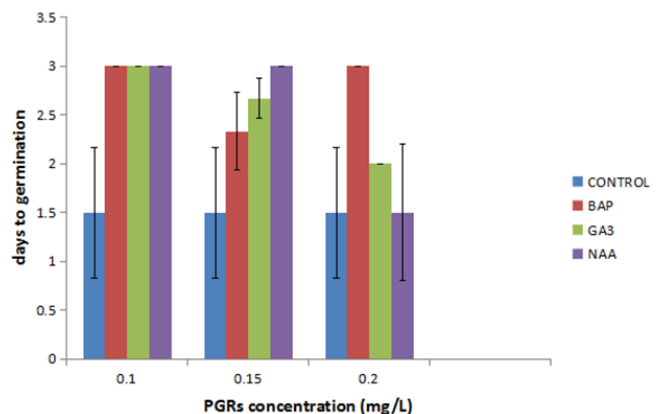


Fig. i: Effects of BAP, GA₃ and NAA on Days to Germination

Best vigor was observed in media treated with 0.20 mg/L BAP, followed by 0.15mg/L NAA. Control and media supplemented with 0.15mg/L GA3, 0.20mg/L GA3 and 0.20mg/L NAA showed least vigor followed by media treated with 0.1 BAP, 0.15 BAP and 0.15 NAA (Fig. ii).

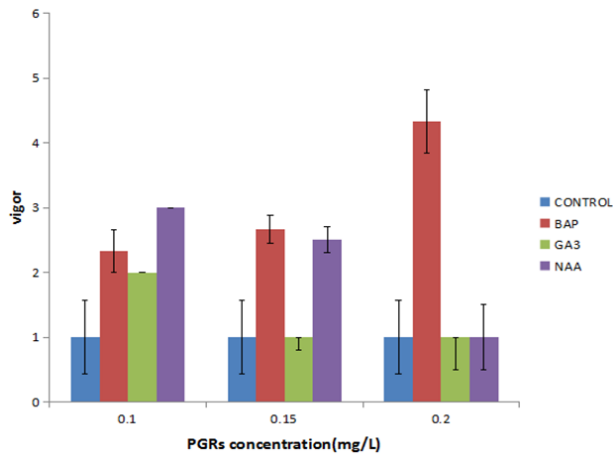


Fig. ii: Effects of BAP, GA3 and NAA on Vigor

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

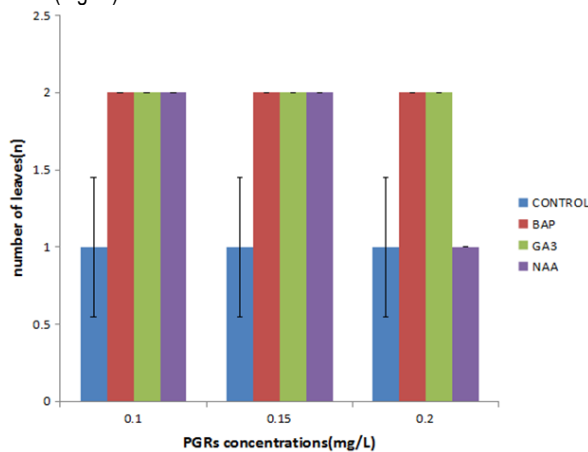


Fig. iii: Effects of BAP, GA3 and NAA on Number of Leaves

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

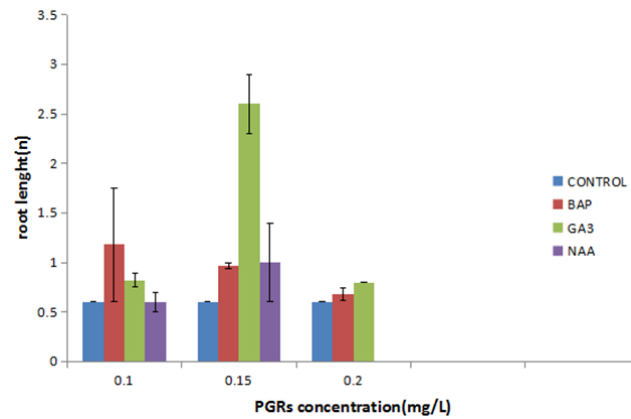


Fig. iv: Effects of BAP, GA3 and NAA on Root Length

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

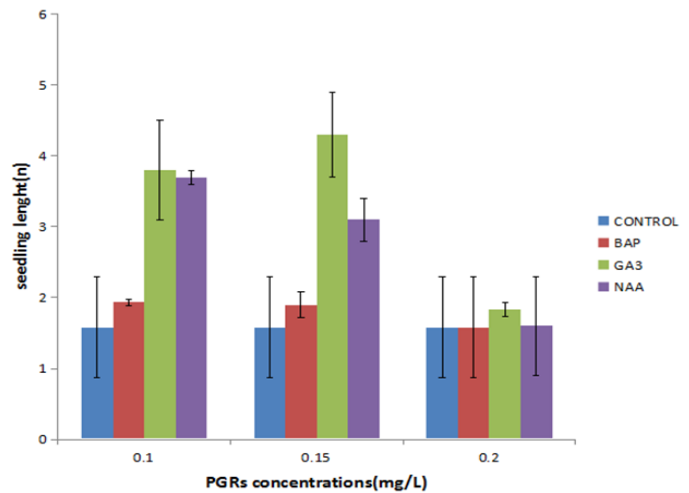


Fig. v: Effects of BAP, GA3 and NAA on Seedling Height

DISCUSSION

The early germination recorded in media treated with NAA 0.02mg/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 unguilata* 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 ± 0.54^9 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 Jamaledine (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 Jayabalan, 2005).

Maximum number of leaves were 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 paradisiacal. At high concentration are inhibitory while low concentration is stimulatory (Syed, 2001).

The highest shoot length recorded in media treated with 0.15 mg/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 0.15 mg/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 the *in vitro* propagation of *Spinachia oleraceali*. A healthy rooting system is vital for tissue culture plantlet survival and rapid adaptation from the *In vitro* micro environment to glass house condition by Taha (2010). This is contrary to the findings of Bakrim *et al.*, (2007) who reported that NAA inhibited root elongation in tomato.

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 basipolarly. 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 0.15 mg/L GA₃ and 0.2mg/l GA₃ and NAA. However, 0.15 mg/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.

Acknowledgements

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.

REFERENCES

- Amali P., Ramakismacomma M., Kingsley, S., and Ignacimuths, S. (2017). *Plant Cell Biotechnology and Molecular Biology* 15 (3&4):118-126)
- Bakrim, A., Lamhamdi, M., Sayah, F. and Chibi, F. (2007). Effects of Plant Hormones and 20-hydroxyecdysone on Tomato (*Lycopersicon esculentum*) Seed germination and Seedling growth. *African Journal of Biotechnology* Vol. 6(24):2792-2802.
- 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.
- Brand, H.M and Lineberger, R.D(1992) Micropropagation of American Sweetgum (Liquidambar styracifina L). In: Y.P.S Bajaj (Ed). Biotechnology in Agriculture and Forestry, Vol.18. High-tech and micropropagation II. Springer, Berlin. Pp 3-24.
- Cato S. C., Macedo W. R., Peres L. E. P. and de C e Castro, P. R. (2013). Sinergism Among Auxins, Gibberellins and Cytokinins In Tomato Cv. Micro-Tom. *Horticultura Brasileira* 31: 549-553.
- Curtis N.B., Michael J.H., and Dallas P. (2017). Interference of redroot pigweed (*Amaranthus retroflexus*) Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean". *Weed Science*,51(1): 37-43
- Gibson, M S.(1980) Measurement of Vigor in Seeds or Seedlings. Sugarbeet Research and Extension Reports, Volume 11, pp 143 – 147.
- Graham, L.E. .Graham, J.M., Wilcox. L.W (2006) Plant Biology. Second Edition.Sheri L. Snavelly. Pearson Education, inc. United State of Americaq. Pp670.
- Hamish, A.c and Sue, E.(1998) Plant Science Culture. BIOS Scientific Publishers limited. Oxford, London. pp157
- He, H., Cai, Y., Sun, M. and Corke, H., 2002). Extraction and Purification of Squalene from Amaranthus Grain". *Journal of Agricultural and Food Chemistry*, 0 (2): 368-372.
- Jamaledine, 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.
- Kalimuthu K, Vijayakumar S, Senthilkumar R. (2010). Antimicrobial activity of the biodiesel plant, *Jatropha curcas*. *International Journal of Pharma and Bio Sciences*, 1(3):1-5.
- Murashige, T. and Skoog, F. (1962) *A Revised Medium for Rapid*

- Growth And Bioassays With Tobacco Tissue Cultures Plant Physiology*, 15:473-497
- 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.
- RHS A-Z Encyclopedia Of Garden Plants. Dorling Kindersley. 2008. p. 1136 Juan; et al. (2007). "Electrophoretic characterisation Of Amaranthus L. seed proteins and its systematic implication". *Botanical Journal of the Linnean Society*, 55 : 57–63.
- Rocio Juan, Julio Pastor, Manuel Alaiz and Javier Vioque (2007). Electrophoretic characterisation of Amaranthus L. seed proteins and its systematic implication. *Botanical Journal of the Linnean Society*, 155 : 57–63.
- Salunke, S.B., Qudrathullah, M.D., Bhavsar, C.M. and Deogadkar, B.B. (1999). *Physical and Agrochemistry Nirali Prakashan*, Pune (2nd Edition) Pp167-190
- 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.
- Syed, S. M. N. (2001). *Plant Growth Hormones: Growth Promoters and Inhibitors*. Nuclear Institute of Agriculture, Tando Jam, Pakistan. Pp40.
- 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.
- Taha R. S., Vahid S., Darioush R., Mahdi E., Javad G., and Roya M., C.(2010). The effect of plant growth regulators, explants and cultivars on spinach (*Spinacia oleracea* L.) tissue culture. *African Journal of Biotechnology*. 9(27) pp. 4179-4185
- 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). *In vitro* regeneration of *Artemisia annua* (Wormwood) using seed explants. *The International Journal of Biotechnology*, 2(11):171-181.
- Tahir, S M, Usman I S, Katung, M D and Ishiyaku, M F (2014). *In Situ* Germination and Early Seedling Growth of Wormwood (*Artemisia annua* L.) *American Journal of Plant Sciences*. 5:1694-1701.
- Tahir, S.M., Dauda, J.T, H, Ibrahim F. Ahmed, S. Yakubu and A. M, Dogara. (2015). Effect of varying Concentrations of Benzleaminopurine(BAP) on the *in vitro* Multiplication of Pineapple (*Anana scomosus* L.) *Katsina Journal Natural and Applied Sciences*.4 (2)146-149. ISSN: 2141-0755.
- Tahir, S.M, Samaila A. A. and Abdulrahman M.D (2019).Studies On the Effects Of 6-Benzylaminopurine And 1-Naphthaleneacetic Acid On The *In Vitro* Regeneration Of Okra (*Abelmoschus Esculentus* L.). *Science World Journal*. 14(1) 119-128.
- Taylor, O.J., Green, N.P.O, and Stout, G.W (1997). *Biological Science*. Cambridge University Press. United Kingdom, Pp984
- Thomas, T.H., (1989).Plant Growth Hormones. *Journal of Plant Growth Regulators*.1 8:255
- Thorpe, T. A. (2007). History of plant tissue culture. *Molecular Biotechnology*. 37, 169–180.
- Tamas, I. A., (1987). In: P.J., Davies, (Ed). *Plant Hormones and Their Role in Plant Growth and Development*.Boston: Martinus Nijhoff.Pp 393.
- Umar, A. (2015). *Effects of Cytokinin and Auxin on the in vitro regeneration of vigna ugulata* (L.) walp. Pp20-24.
- Vasil, I.K. and Vasil, V. (1972) *Totipotency and Embryogenesis in plant cell and tissue cultures In vitro*, 8:117–127.
- Villamor, C.C., 2010. Influence of media strength and sources of nitrogen on micropropagation of ginger, *Zingiber officinale* Rosc. *E. International Science Research. Journal*, 2: 150-155.
- Wickson, M. and Thimann, K.V. (1958). The Antagonism of Auxin and Kinetin in Apical Dominance. *Physiologia Plantaru*. 11:62-74