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Effect of different auxins and cytokinins in callus induction and shoot regeneration in banana (*Musa paradisiaca* L.) variety Udhayam under *in vitro* condition

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Abstract
The experiment was pursued in Tissue Culture Laboratory of Department of Horticulture in Sardar Vallabhbhai Patel University of Agriculture & Technology Meerut during 2016-17 on Udhayam variety of Banana. MS media were prepared. The minimum time of callus induction (24.41 days) was observed in treatment 2,4-D 4.00 mgl^{-1}; while maximum (39.42 days) was noted under control. With the combination of BAP and Kinetin, the earliest shoot initiation (9.87 days) was noted under BAP 4.00 mgl^{-1} + Kinetin 2.00 mgl^{-1}. The minimum days taken for shoot develop (11.47 days) was noted under BAP 4.00 mgl^{-1} + Kinetin 2.00 mgl^{-1}. After, 40 and 60 days maximum number of shoots obtained (5.40) and (71.10) were noted under the same treatment of BAP 4.00 mgl^{-1} + Kinetin 2.00 mgl^{-1}. Maximum percentage of developed shoots obtained (80.95%) was noted under the same treatment of BAP and Kinetin. Maximum shoot length obtained (4.26cm), (5.03cm) and (6.10cm) after 28, 35 and 42 days under the treatment of BAP 4.00 mgl^{-1} + Kinetin 2.00 mgl^{-1}. Viewing above observations it is concluded that 2,4-D 4.00 mgl^{-1} showed better performance on accordance of callus induction while BAP 4.00 mgl-1 + Kinetin 2.00mgl-1 showed better performance on accordance of shoot regeneration.

Keywords: Explant (sword sucker), Banana, Micropropagation, auxin, kinetin, cytokinin, Callus

Introduction
Banana, the fourth largest fruit crop of the world, is a general term referring to a type of fruit or herbaceous plant belonging to Kingdom Plantae, Family Musaceae, of the order Zingiberales and Genus *Musa*. It is native to the tropical region of Southeast Asia. Bananas are likely to have been first domesticated in Papua New Guinea. *Musa* germplasms comprising More than 600 types of wild form as well as cultivated species are reported all over the world. Modern edible varieties have evolved from the two species – *Musa acuminata* and *Musa balbisiana* and their natural hybrids, originally found in the rain forests of South East Asia. *Musa paradisica* is a hybrid of the two species. Banana is cultivated throughout the warm tropical regions of the world between 30º N and 30º S of the equator i.e., it is produced in tropical and subtropical regions of the developing countries (Gebeyehu, 2013) [9]. Banana fruit covers an area of 8.41 million hectare, producing 29.13 million tonnes with a productivity of 14.31 MT/ha during the year 2015-16. In India, Tamil Nadu ranks first in production followed by Gujarat Andhra Pradesh and Uttar Pradesh. However, the productivity was recorded highest in Madhya Pradesh followed by Gujarat, Maharashtra and Tamil Nadu. Banana contributes 32.30 per cent to total production in India. (Review Committee, 16.05.2017., Indian Horticulture N H B Data base 2015-16). Banana is triploid and it is vegetatively propagated through suckers which are the rhizome cut off from the mother plant (Ali *et al*., 2011) [2]. There is need of production of true to the type of plantlets in huge number for commercial cultivation of banana. Through callusing of numerous plantlets can be produced through use of auxins and cytokinins under *in vitro* condition.

Material and Methods
The experiment on micropropation an banana variety Udhayam was conducted in Tissue Culture Laboratory of Department of Horticulture at Sardar Vallabhbhai Patel University of Agriculture & Technology Meerut (U.P.), India during 2016-17.

The Murashige and Skoog 1962 (MS) basal media was prepared with growth regulators such as auxin (2,4-D) and cytokinins (BAP, Kinetin) according to the treatment requirement. Firstly the stock solution of MS media and plant growth regulators (PGRs) as BAP, Kinetin and 2,4-D. All PGRs stock solutions were prepared by weighing the required amount of the chemicals using digital balance and dissolving them in sterilized double distilled water. To prepare 1 liter of MS media, 100 ml of stock solution of macro-nutrients (stock A), 20 ml of stock solution of micro-nutrients (stock B), 10 ml of stock solution of Fe-EDTA (stock C), 100 mg/l of myo-inositol and 20 ml of each of the stock solution of vitamins were added to 1 litre volumetric conical flask 500 ml of distilled water was added. Sucrose @15g per litre, agar powder as gelling agent @ 8 g per litre were added. Then, volume of 1000 ml was maintained through double distilled water. The pH of the medium was adjusted to 5.8 using a digital pH meter with addition either of 1N NaOH or 1N HCl. Different concentrations of growth regulators as required were added either in single or in different combination to this solution and were mixed thoroughly. The specific solutions were boiled under water bath just to dissolve the whole ingradients well. Then they were cooled down and 15 ml each solution was poured separately into sterilized culture containers and they were closed with lid. Then the whole culture containers filled with media were autoclaved with 121.6°C at 15 psi for 20 minutes. For callus induction sword sucker of banana was used as explant in MS media supplemented with 2, 4-D alone (2.00, 3.00, 4.00, 5.00, 6.00, mgl^{-1}) and BAP alone (2.00, 4.00, 6.00, mgl^{-1}) and the different combinations of BAP and Kinetin (2.00+1.50, 2.00+2.00, 2.00+2.50, 4.00 + 1.50, 4.00 + 2.00, 4.00 + 2.50, and 6.00+1.50, 6.00+2.00, 6.00+2.50 mgl^{-1}) were used. Aseptic conditions were maintained throughout the experiment. The laminar air-flow chambers. Its floor is sprayed with spirit. The cleaned media vessels, scalpel, forceps, petri-plates and blade, etc. were autoclaved, before use and kept on the floor of chamber. Switched on the UV-light (Ultra Violet light) for 30 minutes before culturing, then off the UV light and on the laminar air-flow. Scalpels and forceps were dipped in alcohol and flame sterilized regularly during culturing. Hands were also sterilized by wiping with 70 per cent ethyl alcohol. Other required materials like distilled water, glass plate, Petri dish, etc. were sterilized in an autoclave following method of media sterilization. Explants were washed thoroughly under running tap water for about half an hour to remove the soil and dust particles from the plants. Then they were kept in 70 per cent ethyl alcohol for a period of 5 minutes. After that they were washed with double distilled water stirring well. This process was applied five times just to wash out the adhering chemicals on the surface of the explants. The explants were prepared carefully under aseptic condition inside the laminar flow cabinet. After inoculation the test tube was covered and sealed with Parafilm. The prepared culture test tubes after inoculation were kept in culture room at 25±2 °C for callusing. The explants incubated for shoot induction per proliferation were maintained at under light intensity of 30.0 µm $dm^{-1}s^{-1}$ illuminated with white light fluorescence tubes, 25±2 °C temperature, humidity at 65 per cent and photoperiod (2000-3000 lux) of 16 hours light and 8 hours dark in culture room. Each treatment combination was arranged in factorial arrangement in a Completely Randomized Design (CRD), the experiment for callusing one-way ANOVA whereas the experiment for shooting were arranged in two-way ANOVA.

Results and Discussion:

Time of callus induction (days) by 2, 4-D alone:

The effect of different concentrations of 2,4-D for callus induction was used under MS medium. Significant increase in average callus induction (days) in sterilized explants was recorded from 24.41 to 39.42 days after inoculation. It showed that significantly minimum time of callus induction (24.41 days) was observed in treatment 2,4-D 4.00 mgl^{-1} followed by 26.76, 27.90 and 28.73 days with the treatments of 2,4-D 3 mgl^{-1}, 6 mgl^{-1} and 2 mgl^{-1}; while the maximum (39.42 days) was noted under control; however, it was at par with 1% of Critical Difference (Table 01). The same pattern of observations were recorded by Darvari *et al.* (2010) [8]; Rashid *et al.* (2012) [17]; Kumar *et al.* (2013) [14]; Sultan, *et al.* (2011) [22] and Jafari *et al.* (2011) [12].

Table 1: Effect of different concentrations of 2,4-D with MS media on time of callus induction inoculation(days) in banana cv. Udhayam

Treatments	Time of callus induction (days)
2, 4-D 0.00	39.420 a
2, 4-D 2.00	28.730 bc
2, 4-D 3.00	26.760 bc
2, 4-D 4.00	24.410 c
2, 4-D 5.00	29.657 b
2, 4-D 6.00	27.990 bc
Gen. Mean	**29.494 *****
C.V.	9.127
S.E.M.	1.554
C.D.@ 5%	4.789
C.D.@ 1%	6.714

Significance Levels * = <.05, ** = <.01 & *** = <.001

Proliferated callus in banana cultivar Udhayam on MS media supplemented with 2.4-D.

***In vitro* establishment of shoot:**

Time of shoot initiation:

Table 02: Effect of cytokinins on time of shoot initiation after establishment of culture banana cv. Udhayam under half MS medium

Treatments	Time of shoot initiation (days)			
	BAP 2.00	**BAP 4.00**	**BAP 6.00**	**Pooled**
Kinetin 0.00	17.713	14.640	16.923	16.426 a
Kinetin 1.50	13.893	11.683	13.963	13.180 b
Kinetin 2.00	13.980	9.873	13.817	12.557 b
Kinetin 2.50	14.593	12.913	14.340	13.949 b
Gen. Mean	**15.045**	**12.277**	**14.761**	**14.028 ****
C. V.	16.774	15.789	13.162	15.345
S. E. M	1.457	1.119	1.122	0.718
C.D. @ 5%	-	-	-	2.094
C.D. @ 1%	-	-	-	2.838

Significance Levels * = <.05, ** = <.01 & *** = <.001

Maximum time of shoot initiation from the established culture (17.71 days) was noted under the treatment of BAP 2.00 mgl^{-1} followed by 16.92, 14.64 and 14.59 days with the treatments of BAP 6.00 mgl^{-1}, 4.00 mgl^{-1} and BAP 2.00 mgl^{-1} + Kinetin 2.50 mgl^{-1}; while the minimum (9.87 days) was under BAP 4.00 mgl^{-1} + Kinetin 2.00 mgl^{-1}. A critical observation was recorded as the maximum time of shoot initiation in the culture (15.04 days) was recorded under the treatment of BAP 2.00 mgl^{-1}, while the minimum (12.27 days) was under BAP 4.00 mgl^{-1}. A minute observation was recorded as the maximum time of shoot initiation in the culture (16.42 days) was noted under control, while the minimum (12.55 days) was under Kinetin 2.00 mgl^{-1} (Table 02). The same pattern of investigations were carried out by different workers in different parts of the world as Amin *et al*., (2009) [3]; Azam *et al.* (2010) [4] reported multiple shoot cultures from shoot tip explants of Bari-1(AAA) implanted in semisolid MS media fortified with different concentrations of BAP. Rate of shoot proliferation increased when MS media was enriched with 2.0 mgl^{-1} BAP and 1.0 mgl^{-1} Kinetin to the medium increased shoot elongation and several time of the stimulated growth and shoots, respectively.

***In vitro* shoot induction in banana cv. Udhayam on half MS media supplemented with BAP and Kinetin.**

Days taken for shoot development

Table 03: Effect of cytokinins on days taken for shoot development of banana cv. Udhayam under half MS medium

Treatments	Days taken for shoot development			
	BAP 2.00	**BAP 4.00**	**BAP 6.00**	**Pooled**
Kinetin 0.00	21.690 a	18.180 a	24.167 a	21.346 a
Kinetin 1.50	12.400 c	12.257 bc	13.597 c	12.751 d
Kinetin 2.00	14.373 b	11.477 c	16.470 b	14.107 c
Kinetin 2.50	16.023 b	15.253 ab	17.800 b	16.359 b
Gen. Mean	**16.122 *****	**14.292 ****	**18.008 *****	**16.141 *****
C. V.	6.148	11.450	6.750	8.108
S. E. M	0.572	0.945	0.702	0.436
C.D. @ 5%	1.866	3.081	2.289	1.273
C.D. @ 1%	2.715	4.483	3.330	1.725

Significance Levels * = <.05, ** = <.01 & *** = <.001

Maximum time of shoot development from the established culture (24.16 days) was noted under the treatment of BAP 6.00 mgl^{-1} followed by 21.69, 18.18 and 17.80 days with the treatments of BAP 2.00 mgl^{-1}, 4.00 mgl^{-1} and BAP 6.00 mgl^{-1} + Kinetin 2.50 mgl^{-1}; while the minimum (11.47 days) was noted under BAP 4.00 mgl^{-1} + Kinetin 2.00 mgl^{-1}. A critical observation was recorded as the maximum time of shoot development in the culture (18.00 days) was recorded under the treatment of BAP 6.00 mgl^{-1}, while the minimum (14.29 days) was observed under BAP 4.00 mgl^{-1}. So, it was concluded that combination of Kinetin with BAP was more effective as compared to control for shoot development of the explants in the culture under half MS media (Table 03). The result is similar to the findings of Buah *et al.,* (2010) [6]; Jafari *et al.,* (2010) [11]; Reddy *et al*., (2014) [18]; Lohidas *et al.,* (2015) [15] and Rezaul *et al.* (2009) [19].

Number of shoot per explants after 60 days:

Table 04: Effect of cytokinins on number of shoots per explant after 60 days (8 weeks) of banana cv. Udhayam under half MS medium

Treatments	Number of shoots / explants			
	BAP 2.00	**BAP 4.00**	**BAP 6.00**	**Pooled**
Kinetin 0.00	35.867 c	55.633 c	32.500 c	41.333 c
Kinetin 1.50	61.967 ab	64.333 b	61.433 a	62.578 a
Kinetin 2.00	65.833 a	71.100 a	57.267 b	64.733 a
Kinetin 2.50	58.267 b	63.733 b	57.433 b	59.811 b
Gen. Mean	**55.483 *****	**63.700 ****	**52.158 *****	**57.114 *****
C. V.	5.849	5.277	1.142	4.761
S. E. M	1.874	1.941	0.344	0.906
C.D. @ 5%	6.110	6.329	1.122	2.646
C.D. @ 1%	8.891	9.209	1.632	3.586

Significance Levels * = <.05, ** = <.01 & *** = <.001

Maximum number of shoots obtained from the established culture (71.10) was noted under the treatment of BAP 4.00 mgl^{-1} + Kinetin 2.00 mgl^{-1} followed by 65.83, 64.33 and 63.73 with the treatments of BAP 2.00 mgl^{-1} + Kinetin 2.00 mgl^{-1}, BAP 4.00 mgl^{-1} + Kinetin 1.50 mgl^{-1} and BAP 4.00 mgl^{-1} + Kinetin 2.50 mgl^{-1}; while the minimum (32.50) was noted under BAP 6.00 mgl^{-1} alone in half MS medium. A critical observation was recorded as the maximum number of shoots in the culture (63.70) was recorded under the treatment of BAP 4.00 mgl^{-1}, while the minimum (52.15) was observed under BAP 6.00 mgl^{-1} (Table 04). Similar results were reported by Abdelhamid *et al.* (2008) [1] and Khaldun *et al.* (2007) [13].

Per cent of shoot development:

Table 05: Effect of cytokinins on shoot development percent of banana cv. Udhayam under half MS medium

Treatments	Per cent of shoot development			
	BAP 2.00	**BAP 4.00**	**BAP 6.00**	**Pooled**
Kinetin 0.00	42.287 c	64.557 b	36.273 c	47.706 c
Kinetin 1.50	75.127 a	72.130 ab	73.757 a	73.671 a
Kinetin 2.00	72.373 ab	80.950 a	69.983 ab	74.436 a
Kinetin 2.50	68.603 b	73.160 ab	66.307 b	69.357 b
Gen. Mean	**64.597 *****	**72.699 ***	**61.580 *****	**66.292 *****
C. V.	3.813	7.317	3.400	5.421
S. E. M	1.422	3.071	1.209	1.198
C.D. @ 5%	4.637	10.015	3.943	3.497
C.D. @ 1%	6.748	14.573	5.737	4.738

Significance Levels * = <.05, ** = <.01 & *** = <.001

Maximum (80.95%) of developed shoots obtained from the established culture was noted under the treatment of BAP 4.00 mgl^{-1} + Kinetin 2.00 mgl^{-1} followed by 75.12, 73.75 and 73.16 with the treatments of BAP 2.00 mgl^{-1} + Kinetin1.50mgl^{-1}, BAP 6.00 mgl^{-1} + Kinetin 1.5 mgl^{-1} and BAP 4.00 mgl^{-1} + Kinetin2.50 mgl^{-1}; while the minimum (36.27%) was noted under BAP 6.00 mgl^{-1} alone in half MS medium. A critical observation was recorded as the maximum developed shoots per cent in the culture (72.69) was recorded under the treatment of BAP 4.00 $mgl^{-1,}$ while the minimum (61.58) was observed under BAP 6.00 mgl^{-1} (Table 05). The result is similar to the findings of Buah *et al.,* (2010) [6]; Jafari *et al.,* (2010) [11]; Choudhary *et al.,* (2013) [7]; Reddy *et al*.,

(2014) [18]; Lohidas *et al.,* (2015) [15]; Govindaraju *et al.* (2012) [10] and Sazedur *et al.,* (2013) [20].

Shoot length after 28 and 42 days

Table 06: Effect of cytokinins on shoot length after 28 days (4weeks) of culture establishment of banana cv. Udhayam under half MS medium

Treatments	Length of shoot (cm)			
	BAP 2.00	**BAP 4.00**	**BAP 6.00**	**Pooled**
Kinetin 0.00	2.033 b	2.367 b	2.300	2.233 b
Kinetin 1.50	3.500 a	3.167 b	2.867	3.178 a
Kinetin 2.00	3.000 a	4.267 a	2.833	3.367 a
Kinetin 2.50	3.033 a	3.267 b	3.133	3.144 a
Gen. Mean	**2.892 ***	**3.267 ***	**2.783**	**2.981 *****
C. V.	15.336	15.931	12.954	14.973
S. E. M	0.256	0.300	0.208	0.149
C.D. @ 5%	0.835	0.980	-	0.434
C.D. @ 1%	1.215	1.426	-	0.588

Significance Levels * = <.05, ** = <.01 & *** = <.001

The effect of different combination concentrations BAP with Kinetin and BAP alone were used under half MS media. After 28 days, maximum shoot length obtained from the established culture (4.26cm) was noted under half MS medium treated with BAP 4.00 mgl^{-1} + Kinetin 2.00 mgl^{-1} followed by 3.50, 3.26 and 3.16 with the treatments of BAP 2.00 mgl^{-1} + Kinetin 1.50 mgl^{-1}, BAP 4.00 mgl^{-1} + Kinetin 2.50 mgl^{-1} and BAP 4.00 mgl^{-1} + Kinetin 1.50 mgl^{-1}; while the minimum (2.03cm) was noted under BAP 2.00 mgl^{-1} alone. A critical observation was recorded as the maximum shoot length obtained in the culture (3.26cm) was recorded under the treatment of BAP 4.00 mgl^{-1}; while the minimum (2.78cm) was observed under BAP 6.00 mgl^{-1} (Table 06).

Table 7: Effect of cytokinins on shoot length after 42 days (6weeks) of culturet establishment of banana cv. Udhayam under half MS medium

Treatments	Length of shoot (cm)			
	BAP 2.00	**BAP 4.00**	**BAP 6.00**	**Pooled**
Kinetin 0.00	3.867 c	4.067 c	3.200 c	3.711 c
Kinetin 1.50	5.933 a	5.600 ab	5.533 a	5.689 a
Kinetin 2.00	4.867 b	6.100 a	5.000 ab	5.322 a
Kinetin 2.50	4.367 bc	4.900 bc	4.300 b	4.522 b
Gen. Mean	**4.758 ****	**5.167 ****	**4.508 ****	**4.811 *****
C. V.	8.428	9.979	10.124	9.563
S. E. M	0.232	0.298	0.264	0.153
C.D. @ 5%	0.755	0.971	0.859	0.448
C.D. @ 1%	1.099	1.413	1.250	0.607

Significance Levels * = <.05, ** = <.01 & *** = <.001

Finally after 42 days, shoot length was increased under half MS media, the maximum shoot length obtained from the established culture (6.10cm) was noted under the treatment of BAP 4.00 mgl^{-1} + Kinetin 2.00 mgl^{-1} followed by 5.93, 5.60 and 5.53 with the treatments of BAP 2.00 mgl^{-1} + Kinetin 1.50 mgl^{-1}, BAP 4.00 mgl^{-1} + Kinetin 1.50 mgl^{-1} and BAP 6.00 mgl^{-1} + Kinetin 1.50 mgl^{-1}; while the minimum (3.20cm) was noted under BAP 6.00 mgl^{-1} alone. A critical observation was recorded as the maximum shoot length obtained in the culture (5.16cm) was recorded under the treatment of BAP 4.00 mgl^{-1}; while the minimum (4.50cm) was observed under BAP 6.00 mgl^{-1}. A minute observation was recorded as the maximum shoot length obtained in the culture (5.68cm) was noted under Kinetin 1.50 mgl^{-1}; while the minimum (3.71cm) was noted under control. So, it was observed that combination of Kinetin with BAP was more superior as compared to control for production of shoots from the explants in the culture under half MS medium (Table 07). The result is similar to the findings of Buah *et al.,* (2010) [6]; Jafari *et al.,* (2010) [11]; Choudhary *et al.,* (2013) [7], Reddy *et al.,* (2014) [18] and Lohidas *et al.,* (2015) [15]; Strosse *et al.* (2004) [21] reported that the rate of shoot multiplication depends both on the cytokinin concentration and the genotype.

References

1. Abdelhamid MH, Taha RM. Effect of benzylamino purine (BAP) on *in vitro* proliferation and growth of pineapple (*Ananas Comosus* L.) Merr. cv. smooth cayenne. Journal of Applied Sciences. 2008; 8:80-85.
2. Ali A, Sajid A, Naveed NH, Abdul M, Asif S, Umair AK, Fisal IJ *et al*. Initiation, proliferation and development of micro-propagation system for mass scale production of banana through meristem culture. African Journal of Biotechnology. 2011; 10(70):15731-15738.
3. Amin M, Karim MR, Amin MR, Rahman S, Mamun ANM. *In vitro* micropropagation of banana (*Musa spp*.). Bangladesh Journal of Agriculture Research. 2009; 34(4):645-659.
4. Azam FMS, Islam S, Rahmatullah M, Zaman A. Clonal propagation of *Musa spp.* cultivar BARI-1 (AAA genome, sapientum subgroup). *Proc. IC on Banana & Plantain in Africa. Acta Hort.* 2010, 879.
5. Bandurski K, Raj S, Rajendran R. Effect of Benzylamino purine (BAP) pulsing on *in vitro* shoot multiplication of *Musa acuminata* (banana) cv. Berangan. African Journal of Biotechnology. 1995; 10(13):2446-2450.
6. Buah JN, Danso E, Taah KJ, Abole EA, Bediako EA, Asiedu J *et al*. The effects of different concentrations cytokinins on the *in vitro* multiplication of plantain (*Musa* sp.). Asian Network for Scientific Information. 2010; 9(3):343-347.
7. Choudhary D, Kajla S, Poonia AK, Brar B, Surekha, Duhan JS. Comparative study of various growth regulators on *in vitro* multiplication of commercial cultivar of banana Grand Naine. Annals of Biology, 2013; 29(3):288-293.
8. Darvari FM, Sariah M, Paud MP, Maziah M. Micropropagation of some Malaysian banana and plantain (*Musa spp.)* cultivars using male flowers. African Journal of Biotechnology. 2010; 9:2360-2366.
9. Gebeyehu A. Effect of different combinations of 6-Benzylamino purine and Naphthalene acetic acid on multiple shoot proliferation of plantain (*Musa spp*) cv. Matoke from meristem derived explants. African Journal of Biotechnology. 2013; 12(7):709-719.
10. Govindaraju S, Saravanan J, Jayanthi B, Nancy D, Arulselvi I. *In vitro* propagation of Banana (*Musa sp* – Rasthali variety) from sword suckers for its commercial production. Research in Plant Biology. 2012; 2(5):2231-5101.
11. Jafari N, Othman RY, Khalid N. Effect of Benzylamino purine (BAP) pulsing on *in vitro* shoot multiplication of *Musa acuminata* (banana) cv. Berangan. African Journal of Biotechnology. 2010; 10(13):2446-2450.
12. Jafari N, Othman RY, Khalid N. Effect of Benzylamino purine (BAP) pulsing on *in vitro* shoot multiplication of *Musa acuminata* (banana) cv. Berangan. African Journal of Biotechnology. 2011; 10(13):2446-2450.
13. Khaldun ABM, Haque MS, Uddin MS, Billah MM. Effect of BAP and IAA on *in-vitro* plantlet regeneration

of local banana *(Musa sp.)* cultivars. International Journal of Sustainable Agricultural Technology. 2007; 3(2):24-29.
14. Kumar D, Anbazhagan M, Rajendran R. *In Vitro* propagation of Banana (*Musa acuminate* L.) cv. Cavandish Dwarf. International Journal of Research in Biomedicine and Biotechnology. 2013; 3(3):44-46.
15. Lohidas J, Sujin D. Effect of growth hormones in the micropropagation of banana cv. Matti. Plant Archives 2015; 15(1):307-314.
16. Rahman MZ, Sharoar MG, Matin MN, Rahman MH, Rahman MM, Islam MR. High frequency plant regeneration of a dessert banana cv. Mehersagar for commercial exploitation. Biotechnol. 2006; 5(3):296-300.
17. Rashid A. Cell physiology and genetics of higher plant 1: CRC Press, Boca Raton Fla. 2012; 1(38):67-107.
18. Reddy D, Suvarna D, Rao M. Effects of 6-Benzylamino purine (6-BAP) on *in vitro* shoot multiplication of Grand Naine (*Musa sp.).* International Journal of Advanced Biotechnology and Research. 2014; l5:36-42.
19. Rezaul KM, Malek MA, Sajia RM, Al-Amin, Rauhul Amin M. *In vitro* propagation of banana. Bangladesh J Agril. Res. 2009; 34(2):269-278.
20. Sazedur R, Nirupam B, Mrhied H, Golam A, Mamum ANK, Rafiuqul I *et al.* Micropropagation of banana (*Musa spp*.) Cv. Agnishwar by *in vitro* shoot tip culture. Int. Res. J Biotech. 2013; 4(4):83-88.
21. Strosse H, Van Den Hauwe I, Panis B. Banana cell and tissue culture review. Banana improvement cellular. Molecular biology and induced mutations. Mol. Boil. 2004; 50:82-88.
22. Sultan Md T, Khan MH, Hakim Md L, Mamun ANK, Morshed Md A, Islam Md R. *In vitro* plant regeneration from male flowers of banana. International Journal of Biosciences. 2011; 1(1):1-11.