IN VITRO PROPAGATION OF BANANA (*Musa* spp.)

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CERTIFICATE

This is to certify that thesis entitled, **"*IN VITRO* PROPAGATION OF BANANA (*Musa* spp.)"** *submitted to the Faculty of* **AGRICULTURE,** *Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* **MASTER OF SCIENCE** in **BIOTECHNOLOGY**, *embodies the result of a piece of bona fide research work carried out by* **FAHIMA KHATUN,** *Registration No.* **08-3045** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Date: December, 2013
Place: Dhaka, Bangladesh

(Homayra Huq)
Associate Professor
Supervisor

DEDICATED TO

MY

BELOVED PARENTS

ABBREVIATIONS AND ACRONYMS

Agril.	:	Agriculture
Biol.	:	Biological
BAP	:	6- Benzyl Amino Purine
BA	:	Benzyladenine
BARI	:	Bangladesh Agricultural Research Institute
BBS	:	Bangladesh Bureau of Statistics
CLBs	:	Cauliflower Flower Like Bodies
Cm	:	Centimeter
CRD	:	Completely Randomized Design
CV.	:	Cultivar
Conc.	:	Concentration
2,4-D	:	2,4- Dichlorophenoxy acetic acid
DAI	:	Days After Inoculation
Dw	:	Distilled water
DMRT	:	Duncan's Multiple Range Test
et al.	:	And others (at elli)
FAO	:	Food and Agricultural Organization
g/L	:	Gram per litre
IAA	:	Indole acetic acid
IBA	:	Indole butyric acid
Int.	:	International
2-ip	:	2-isopentenyladenine
J.	:	Journal
Mol.	:	Molecular
mg/L	:	Milligram per litre
µM	:	Micromole
MS	:	Murashige and Skoog
NAA	:	*a*- Napthalene acetic acid
PGRs	:	Plant Growth Regulators
PP333	:	Paclobutrazol
Res.	:	Research
Sci.	:	Science
TDZ	:	Thidiazuron

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BY

FAHIMA KHATUN

ABSTRACT

The experiment was conducted at the Biotechnology Laboratory, Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka, during October 2013 to August 2014 to investigate effects of benzylaminopurine (BAP) concentrations (1.0, 2.0, 3.0, 4.0 and 5.0 mg/L) and indole-3-butyric acid (IBA) (0.5, 1.0, 1.5, 2.0 and 2.5 mg/L) for *in vitro* regeneration of Sagar and Sabri Banana varieties using shoot tip explants. The highest response of explants 88% in Sagar and 84% in Sabri were with 4.0 and 5.0 mg/L BAP, whereas in 4.0 mg/L BAP+2.0 mg/L IBA had 85% response in Sagar and 5.0 mg/L BAP+2.5 mg/L IBA had 90% response in Sabri. In both Sagar and Sabri, minimum number of days for shoot initiation (7.80 and 9.40 days), maximum number of shoots per explant (6.80 and 3.40), highest shoot length (8.31 and 6.19 cm), number of leaf (4.24 and 4.49) were observed in 4.0 mg/L and 5.0 mg/L BAP. In contrast, due to combined effect, the highest shoot number per explant (5.60 and 3.40) was in 4.0 mg/L BAP+1.5 mg/L IBA and 5.0 mg/L BAP+2.0 mg/L IBA in Sagar and Sabri. Again, root formation required minimum 7.60 and 8.00 days in 1.5 mg/L IBA for Sagar and Sabri, respectively. The highest number of roots (5.20 per explant) was observed in 0.5 mg/L of IBA for Sagar and that for Sabri was 3.40 per explants observed in 1.5 mg/L of IBA at 6 WAI. In combination both for Sagar and Sabri, the highest number of roots (5.00 and 5.20 per explant) and root length (6.61 and 6.72 cm) were produced by 3.0 mg/L BAP+1.0 mg/L IBA. In natural environment, Sagar variety survived more (94.74%) in soil than Sabri variety (88.89%). Ultimately combined effect of BAP and IBA seemed better than individuals based on average performance of growth parameters and the protocol optimized in the present study could be used *in vitro* rapid propagation of Banana plantlets.

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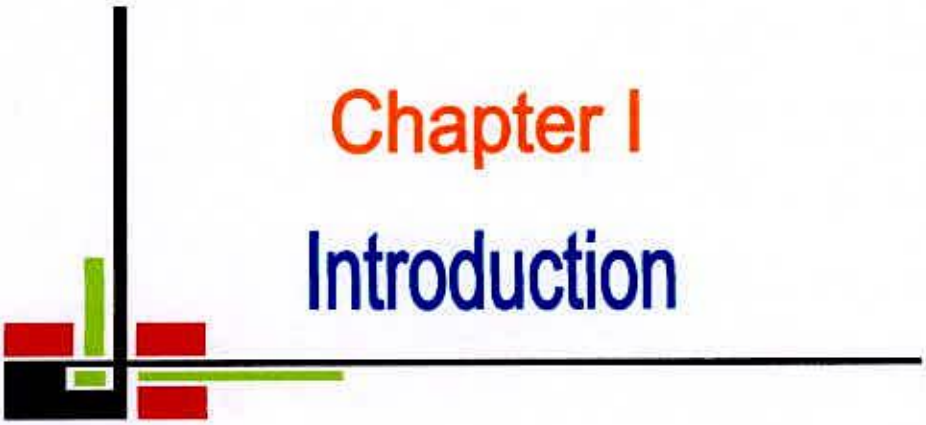


Chapter I

Introduction

CHAPTER I
INTRODUCTION

The Banana *(Musa* sp.) belonging to the family Musaceae is one of the world's most important subsistence crops. It is originated in Malaysia through a complex hybridization process (Novak, 1992). It is the world's oldest cultivated crop (Kumar *et al.*, 2012). In terms of gross value of production, Bananas are the world's fourth most important crop after rice, wheat and maize (CGIAR, 2011). According to the Food and Agriculture Organization, 98 per cent of world's Banana production was derived from developing countries. In international trade, Bananas account for ~22 per cent of world's fresh fruit production and are ranked second most important fruit crop after citrus (FAO, 2010). Presently, Banana is grown in around 150 countries across the world on an area of 4.84 million ha (FAOSTAT, 2011) with an annual production of 92.38 million tons (Guerrero, 2012). They contribute to food security by producing fruit year around and provide incomes to rural populations (Roux *et al.*, 2008).

Banana is being cultivated in tropical and subtropical countries like Indonesia, Thailand, Bangladesh, Vietnam, the Philippines, India, Malaysia, Taiwan, Srilanka and South China, as a major staple food for 400 millions of people. Banana is also a primary food and cash crop for over 30 million people in East Africa (Wanja, 2010).

As a diet, Banana is an affluent source of carbohydrate with calorific value of 67 calories per 100g fruit and is one of the most well liked and widely traded fruits across the world (Emaga *et al.*, 2008; Kumar *et al.*, 2012). It is also rich in vitamins A, B_6, and C, potassium and some minerals, notably phosphorus and calcium. In addition, it has importance for tannin, latex and fiber production. Sabri variety of Banana being free from substances that give rise tri-uric acid is ideal for patients with gout or arthritis.

In our country, Banana is popular for its year round availability, abundant production as well as high acceptability to the consumers. Total estimated production of Banana was 801000 metric tons and cultivated area is 131 acres (BBS, 2012) in the country. This yield is quite low compared to other Banana growing countries of the world.

India alone accounting for 27.43% (26.2 million tonnes) followed by Philippines, producing 9.01 million tones and China, Brazil and Ecuador, with production ranging from 7.19 to 8.21 million tonnes (Singh *et al.*, 2011). The great bulk of Bananas produced in our country are traded and consumed in domestic markets.

There are many cultivars of Banana, such as Amritsagar, Sabri, Champa, Mehersagar, Dudsagar, Kabri, Agniswar, Genasundari, Kanaibashi, Basrai, Binisuta etc. Sabri is a commercial variety. Fruits are medium-sized with a thin peel, ivory yellow in colour, firm in texture, sweet and tasty. Ripe Bananas are one of the most rapidly digested foods. Amritsagar, the widely accepted commercial variety has been declining due to its low yield. Amritsagar and Sabri variety of Banana yield was 13.5 kg and 10.2 kg/bunch, respectively at Trishal, Mymensingh. Different cultivars or varieties produced varying yield in different region of different countries.

Banana and plantain plants are susceptible to a wide range of diseases and pests. Some pests and diseases are highly aggressive, very contagious and easily spread. Once established they are persistent and practically difficult to eradicate (Nelson *et al.*, 2006). As a result, Banana productivity decreases and the yield become very poor. Only 5 to 10 suckers can be obtained from a plant per year in conventional method as, traditional clonal propagation method appears unable to satisfy the increase in demand for disease free and healthy planting materials of Banana. The fungal diseases are the main diseases in the Banana and plantain plantations. A complex of Banana pests such as nematodes and Banana weevil are the major threats to Banana production, causing a yield loss of up to 85% (Musambiyama *et al.*, 2000).

All Banana cultivars in affected areas are susceptible to Banana bacterial wilt (BBW) and the disease is rapidly spread (Heslop - Harrison and Schwarzacher, 2007). It has been found that the BBW is a very destructive disease with an incidence of 70 - 80 % in many plantations and the yield losses of 90 % have been reported on some farms (Agrios, 2005).

As Bananas are parthenocarpic, its traditional propagation is done by small shoots or suckers (four to five suckers per plant) from the parent plant. Multiplication through

this method is slow and leads to the transmission of a number of viral diseases (Bunchy top virus, Banana streak virus) to the consecutive generation. As a result, fruiting is reduced and Banana production has decreased gradually in Bangladesh. The major reason is believed to be unavailability of healthy and virus free suckers as traditional propagation is inadequate to restore the affected farms with healthy germplasm. The high sterility of most cultivated Bananas has historically prevented conventional breeding programs, as it has similarly affected plant propagation. Moreover, the longer time required by Bananas to generate makes it even more difficult to breed them (Sasson, 1997).

Tissue cultured plants grow vigorously, establish more quickly and take a shorter time to bunch emergence and harvest. Tissue culture technique produce 39% higher yield than conventional sword suckers (Farahani *et al.*, 2008). Under Bangladesh conditions, tissue culture derived plantlets of Banana performed better than the conventional sword suckers (Faisal *et al.*, 1998).

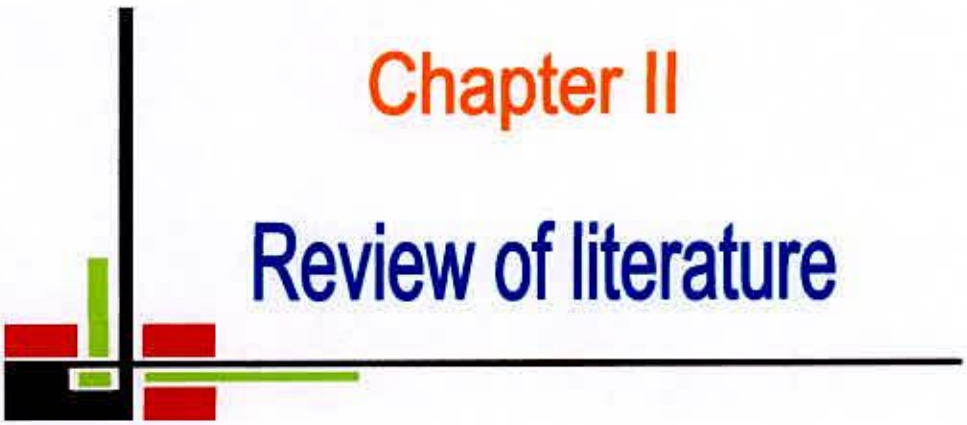
With the increasing demand and vast export potential coupled with the farmers desire to grow *in vitro* propagated Banana on a large area are becoming increasingly important in planting material for rapid multiplication of economically important commercial varieties (Roux *et al.*, 2001; Ray *et al.*, 2006). A large number of uniform disease free plants can be produced from a single plant or even a small plant tissue (explants) showing good genetic potential in this method (Martin *et al.*, 2006) and plant multiplication can be continued throughout the year irrespective of seasonal variation (Rahman *et al.*, 2004).

In tissue culture, plant growth regulators (PGR) are critical media components in determining the developmental pathway of the plant cells. Cytokinin such as benzylaminopurine (BAP) and kinetin are generally known to reduce the apical meristem dominance and induce both axillary and adventitious shoots formation from meristematic explants in Banana (Madhulatha *et al.*, 2004). The effectiveness of BAP over other cytokinins in inducing multiplication of shoot tip cultures has been reported in different cultivars of Bananas (Buah *et al.*, 2010; Farahani *et al.*, 2008; Rahman *et al.*, 2006). BAP has a marked effect in stimulating the growth of axillary and adventitious buds and foliar development of shoot tip cultures (Abeyaratne *et al.*,

2002; Buah *et al.*, 2010). Meanwhile, combinations of BAP with auxins such as indole acetic acid (IAA) or indole-3-butyric acid (IBA) were also used for *in vitro* multiplication of Bananas (Dhed'a *et al.*, 1991; Resmi *et al.*, 2007). BAP is also known to have mutagenic effects at high concentration producing off type plantlets (Bairu *et al.*, 2008).

Generally, cytokinin helps in shoot proliferation and auxins helps in rooting of proliferated shoots. However, the requirement of cytokinin and auxins depends on the variety of Banana and culture conditions (Cronauer and Krikorian, 1984a). Sagar and Sabri varieties play a vital role in our national economy due to its popularity and acceptability to marginal and commercial farmers. To obtain disease free healthy planting materials, development of a protocol for shoot tip culture of Sagar and Sabri, with the optimized concentration of the cytokinin (BAP), auxin (IAA) has been set up as a target in this study. Therefore, the present study was undertaken with the following objectives:

i. To study the individual effect of BAP and IBA on *in vitro* regeneration of Sagar and Sabri varieties of Banana.
ii. To study the combined effect of BAP and IBA on *in vitro* regeneration of Sagar and Sabri varieties of Banana, and
iii. To develop an efficient protocol for *in vitro* rapid propagation of Banana varieties.



Chapter II

Review of literature

CHAPTER II
REVIEW OF LITERATURE

Banana and plantain *(Musa* sp.) are the quick growing fruit crops in Bangladesh. Bananas are generally propagated by suckers. But now-a-days they are also propagated through tissue culture. *In vitro* culture depends on various factors, like composition of media, explants and environmental conditions. e.g. temperature, light, humidity etc. The technique of plant tissue cultures have been developed as a new powerful tool for crop improvement and emphasized wide attention of modern scientists. Many plant breeders of different countries have been employing biotechnological tools for the development of the Bananas, whereas it is very limited in Bangladesh. Related works already performed by different institutes of the world have been reviewed and some of the most relevant literatures are cited here under different headings.

2.1 Concept of Banana Tissue culture

The first report of Banana tissue culture came in early 1970's from Taiwan when Ma and Shii (1972) produced *in vitro* adventitious buds from Banana shoot apex followed by Berg and Bustamante (1974) in Honduras, who used meristem culture combined with thermotherapy for the production of virus free Banana plants. Since then people are working on different aspects of Banana tissue culture as an enabling tool for maximizing Banana production.

2.1.1 Explant

Any plant parts such as shoot apices obtained from parental pseudostem, sucker, peeper, lateral bud or terminal inflorescence used in *in vitro* culture is called explant. A differential *in vitro* response of explants was reported by a number of authors, some of which are reviewed here:

Any part of the Banana plant including pseudostems, suckers, peepers, lateral buds or even small eyes which contain a shoot meristem can be used as explants in TC (Jarret *et al.,* 1985; Vuylsteke and De Langhe, 1985).

Goswami and Handique (2013) carried out an experiment on the explants size response to *in vitro* propagation of Musa (Aaa Group) 'Amritsagar' Musa (Aab Group) 'Malbhog' and Musa (Aab Group) 'Chenichampa' Banana. The effect of three different sizes of explants (5, 10 and 20 mm) on the establishment of Banana in micropropagation was investigated using sword suckers. Initiation media- MS Basal, MS+BAP 0.2 mg/L, MS+BAP 0.3 mg/L and MS+BAP 0.5 mg/L were used to evaluate the explants size response for initiating and establishing Banana cultures *in vitro* condition. The larger explants (20 mm) responded well with regard to survival of explants, days to swelling and greening of explants, emergence of leaf and days to multiple bud initiation under *in vitro* condition as compared to smaller explants.

Rahman *et al.* (2012) observed that micropropagation from shoot tips of one-month-old suckers of six Banana cultivars and subsequent field evaluation for commercial exploitation, were studied. In the second part induction of callus from the male flowers and subsequent somatic embryogenesis were tested. Among the five cultivars male flowers of Sabri were found to show the highest response to callus induction and subsequent embryo formation.

Ahirwar *et al.* (2012) carried out an experiment on a high frequency plantlets regeneration for Banana (*Musa paradisiaca* L.) micropropagation where the shoot tip was inoculated on MS medium containing different concentrations of BAP (0-10.0 mg/L); kinetin (0-10.0 mg/L); and different combinations of BAP (0-10.0 mg/L) and NAA (0.3-0.5 mg/L). The highest frequency of shoot regeneration (52.25%), number of shoots regenerated per explant (3.25) and shoot length (4.69 cm) was observed from shoot tip explants cultured on MS medium supplemented with 5.0 mg/L BAP.

Darvari *et al.* (2010) observed that male inflorescences have potential to be used as explants for rapid micropropagation of *Musa* spp. The male flowers of four Banana cultivars, namely 'Berangan', 'Rastali', 'Nangka' and 'Abu' belonging to three genome types in *Musa* (AAA, AAB, and ABB), were cultured onto Murashige and Skoog (MS) medium which was supplemented with 1.0 mg/L of TDZ, BAP, Kin, 2-ip and Zeatin.

Morais-Lino *et al.* (2008) studied immature male flowers as a explant which generated highly embryogenic cultures and were used for establishment of cell suspension culture and multiplication of secondary somatic embryos. In Banana TC, a sucker was detached from the parent plant and the outside tissue was pared away to get only the growing point of approximately 10 mm^3 revealing TC plants have inherently high level of juvenile vigor which renders them more photosynthetically active compared to plants derived from suckers (Robinson and De Villiers, 2007).

Shoot tip culture is the basic technique for *Musa* propagation (Ma and Shii, 1974; Swamy *et al.*, 1983; Cronauer and Krikorian, 1984a; 1984b; Singh *et al.*, 2004; Pua, 2007). The rate of shoot proliferation is the most important factor of micropropagation (Pua, 2007; Bairu *et al.*, 2008). Plants regenerated from shoot tip culture have shown to perform identically well or even better than those from conventional vegetative propagation under field conditions (Vuylsteke and Ortiz, 1996; Pua, 2007).

Scalp of banana which has been reported by many researchers is consisted of several fleshy bulbous structures producing tiny white tissues and looks like cauliflower (Dhed'a *et al.*, 1991; Villalobos and Garcia, 2008; Sholi *et al.*, 2009) possessing highly proliferating ability as a good source for embryogenesis and providing embryogenic cell suspensions (Dhed'a *et al.*, 1991; Strosse *et al.*, 2004). The establishment of embryogenic cell suspensions from scalps suggests a better choice for plant regeneration as it can be obtained at any growth stage and thus saves the time (Sadik *et al.*, 2007). *In vitro* propagation of Bananas using shoot tips has been reported for many commercial cultivars (Kulkarni *et al.*, 2004, 2006).

Rahaman *et al.* (2004) had also observed hard ball like structure developed from meristem explant in MS media supplemented with 5.0 mg/L BAP. They noticed that single shoot regeneration from meristem explant was thinner than shoot derived from shoot tip. In case of micropropagation of Banana, different types of explants such as offshoots, rhizomes or aerial organs (leaf, pseudostem or fruit) may be used.

2.1.2 Shoot induction of Banana

Morfeine (2014) studied on the effect of sucrose and glucose concentrations on micro-propagation of *Musa* sp.cv. Grand Naine. The results indicated that the 30g/L and 45g/L sucrose and 45g/L and 60g/L glucose had significant increase in micro-propagation of *Musa* sp.

Rahman *et al.* (2013) micropropagated Banana (*Musa* sp.) cv. Agnishwar by *in vitro* shoot tip culture. Shoot tips obtained by removing leaf sheaths from sucker were cultured aseptically in MS medium supplemented with different concentrations of cytokinins viz. 6- BAP, kin, N6 -2iP for multiplication of shoot and auxins viz. IBA, α- NAA for induction of root. Maximum multiplication (95%) was obtained in MS medium containing 4.0 mg/L BAP. The highest average number of shoots for each explant (5.9) was found in MS medium fortified with 4.0 mg/L BAP while maximum elongation of shoot (4.9 cm) was observed in MS medium having 5.0 mg/L BAP.

Iqbal *et al.* (2013) carried out an experiment on the optimization of *in vitro* micropropagation protocol for Banana (*Musa sapientum* L.) under different hormonal concentrations and growth media MS medium supplemented with BAP and IAA (5.0 + 1.0 mg/L, respectively) and 10% CW were found to be most efficient and productive combination for shoot proliferation.

In a study of *in vitro* plant regeneration in Banana (*Musa* sp.) cv. Sabri by using meristematic stem cuttings explant, MS medium supplemented with BAP singly or in combination with auxin, IAA and coconut water was used. Highest percentage of shoot regeneration (90%) and maximum number of shoots (10) per explant were observed when cultured on MS + 4.0 mg/L BAP + 2.0 mg/L IAA + 13% (v/v) coconut water (Huq *et al.*, 2012).

Vora and Jasrai (2012) worked on substitution of synthetic PGR by natural and low cost substitutes. Synthetic cytokinin BA (3.0-5.0 mg/L) was used for *in vitro* shoot multiplication. Sweet-lime juice was found useful as the substitute of such costlier synthetic cytokinin.

Chariya (2012) carried out an experiment on *Musa* sp. where series of media containing different concentration of BAP ranging from 1.0 to 6.0 mg/L were studied. The initiation of shoot was observed after 12 days of inoculation in each media. At subculture level the hardness of the tissue was observed in each shoot. Sprouting of shoot was low in media containing 1.0 mg/L BAP this hormonal concentration. In media containing 3.0 and 4.0 mg/L BAP, sprouted shoot was observed in relatively better developed conditions. The initiation of 2-4 leaves with healthy development of shoot was observed in media containing 5.0 mg/L BAP, while with 6.0 mg/L BAP in media two leaves showing slight healthy shoots was observed. Thus, in general, 5.0 mg/L BAP containing media was sound better at initiation stages of culture growth.

Rahman *et al.* (2012) found that in micropropagation of elite genotypes of banana, the medium with 5.0 mg/L BAP was the most effective for micropropagation. Among the different media formulations, the medium fortified with 4.0 mg/L 2, 4-D + 1.0 mg/L each of IAA, NAA and biotin was found to be the most effective growth regulator formulation for callus induction and growth. Somatic embryo derived plants of all Banana cultivars at maturity exhibited wide range of variation in yield and yield contributing characters.

In an investigation of Bhosale *et al.* (2011), the effect of different concentrations of BAP on shooting in different species of Banana viz. Ardhapuri, Basrai, Shrimanti, were reported. Sword suckers with medium size were inoculated on MS medium supplemented with different concentrations of BAP (3.0 mg/L, 5.0 mg/L, 7.0 mg/L, 9.0 mg/L) cultures were incubated at 25 ± 1^{0}C with a 16 hr photoperiod (2000 lux).

Azam *et al.* (2010) carried out an experiment on the clonal propagation of Banana (*Musa* sp.) cultivar BARI-1 (AAA genome, *sapientum* subgroup). *In vitro* cormlets were formed within 2–3 weeks when meristems were carefully isolated from field-grown plants and after proper sterilization implanted in semisolid MS media fortified with 2.0 mg/L BAP. Rate of shoot proliferation increased considerably with the synergistic effect of BAP and Kinetin. Regeneration of cormlets was geared up and shoot multiplication occurred when MS was enriched with 2.0 mg/L BAP and 1.0

mg/L Kinetin. With the increase of subculture (up to 9th maximum), frequency of shoot proliferation was enhanced. Addition of 0.1 mg/L IBA and 10% coconut water to the medium increased shoot elongation and stimulated growth of the shoots, respectively.

Gitonga *et al.* (2010) studied on the low technology tissue culture materials for initiation and multiplication of Banana plants. In this study, they evaluated a micropropagating protocol for local Banana (*Musa* sp.) (Muunju landrace) in Kenya as an alternative to reduce the unit cost of tissue culture micropropagation.

Darvari *et al.* (2010) conducted an experiment on the micropropagation of some Malaysian Banana and plantain (*Musa* sp.) cultivars using male flowers. The male flowers of four Banana cultivars, namely 'Berangan', 'Rastali', 'Nangka' and 'Abu' belonging to three genome types in *Musa* (AAA, AAB, and ABB), were cultured onto MS medium which was supplemented with 1.0 mg/L of TDZ, BAP, Kin, 2-ip and Zea. The number of shoots was found to significantly increase in both TDZ and BAP treatments, as compared to other cytokinins. TDZ at 0.4, 0.6 and 0.8 mg/L, in particular, appeared to be optimum for shoot induction in 'Berangan- AAA', 'Rastali-AAB' and 'Nangka-AAB' and 'Abu-ABB', respectively. However, all the cultivars showed their highest response to regeneration at 8.0 mg/L of BAP. The number of induced 'CLBs' cluster is dependent on the size of male buds.

Vishwas *et al.* (2010) studied on field performance of *in vitro* propagated Banana plants from 8th and 15th subculture. In this experiment, the 8th subculture plants started growth earlier & grew faster enabling them to intercept more light for photosynthesis than 15th subculture plants. The 8th subculture plants have more uniform growth than 15th subculture with very less variation. This may explain the higher yield in the 8th subculture plants. MS medium supplemented with 6 BAP (3.0 mg/L) was used for initiation and multiplication of shoot.

Karim *et al.* (2009) reported on development of an *in vitro* technique for plant regeneration using meristem-derived plantlets of Banana cv. BARI-l *(Musa* sp.) has

been developed. Highest number of shoot regeneration was noticed on basal media supplemented with 7.5 mg/L BAP + 0.5 mg/L NAA at 30 days after inoculation (DAI). The mean number of shoots significantly reduced when the concentrations of BAP and NAA in the medium was high.

Al-amin *et al.* (2009) investigated the effect of different concentrations of BAP and NAA on virus free plant regeneration and shoot multiplication of Banana cv. BARI Banana-I. The culture meristem first turned brown in color in 4-5 days which grew into a green globular hard coat mass after 30-35 days. From this ball like structure, adventitious plantlets were developed. Low auxin in combination with high cytokinin induces auxilary shoot proliferation (George, *et al.* 2008).

Morais-Lino *et al.* (2008) carried out an experiment on the cell suspension culture and plant regeneration of a Brazilian plantain, cultivar Terra. Five semisolid culture media were tested for differentiation, maturation, somatic embryos germination and for plant regeneration. An average of 558 plants per one milliliter of 5% SCV (settled cell volume) were regenerated in the MS medium, with 11.4 µM IBA and 2.2 µM BAP. Regenerated plants showed a normal development, and no visible somaclonal variation was observed *in vitro*. It is possible to regenerate plants from cell suspensions of plantain Banana cultivar Terra using MS medium supplemented with 11.4 µM of IAA and 2.2 µM of BAP.

Strosse *et al.* (2008) studied the effects of different cytokinins on Banana proliferation. In particular, cytokinin has been found to reduce the dominance of apical meristems and induce axillary shoots, as well as formation of adventitious shoot from meristematic explants (Madhulatha *et al.* 2004).

Lee (2005) tested thidiazuron (TDZ) in the range of 0.002 to 2.0 mg/L for its influence on the proliferation of adventitious buds. TDZ at 0.2 mg/L almost doubled the multiplication rate of Cavendish cultivars in medium containing BA at 4.0 mg/L. The multiplication rate of shoots decreased gradually with increasing numbers of subculture cycles. The addition of paclobutrazol (PP333) (0.5 - 1.0 mg/L) to TDZ (0.1 - 0.2 mg/L) resulted in a further increase of proliferation of buds when compared with medium containing only TDZ, while also suppressing shoot

elongation. The dwarf bud clusters induced in the TDZ + PP333 medium returned to normal shoots after one to two subcultures in medium containing only BA.

Muhammad *et al.* (2004) carried out an experiment on the Banana plantlet production through tissue culture. *In vitro* multiplication of Banana (*Musa* sp.) cv. Basrai was studied. Shoot tips were cultured on MS basal medium supplemented with 5.0 mg/L BAP. Observations were recorded at an interval of four weeks for five subcultures. Evaluations were done at each subculture by counting the number of new shoots produced. Shoot tips coming from different rhizomes behaved differently under *in vitro* conditions. Some being highly productive while others produced less number of shoots. On the average, 124 plants were produced from each shoot tip after five subculturing.

Santos and Rodrigues (2004) investigated the somaclonal variation event on micropropagated *Pacovan* Banana seedling (*Musa* sp. AAB Group). Apex stems were introduced and multiplied *in vitro* using culture media MS, with addition of 2.5 mg/L of BAP. In subsequent subcultures, MS with 4.0 mg/L of BAP was used to induce side buds. Results showed that plants were regenerated with different numbers of subculture (3, 4, 5, 6, 7, 8, and 9). Somaclonal variation occurred from the fifth subculture, and on the 9 subculture a 5.8% variation was observed. The increase of percentage of somaclonal variation, due to a higher number of subcultures, indicates the necessity of protocols for micropropagation specific for each variety to be commercialized.

Habiba *et al.* (2002) reported that the best medium for single shoot development to obtain contamination free culture of the table Bananas *Musa sapientum* cv. Chini, Champa and Amritasagar was MS media supplemented with 4.0 mg/L BAP and 1.0 mg/L Kinetin. Whereas the best medium for shoot multiplication was MS medium fortified with 4.0 mg/L BAP, 2.0 mg/L IAA and 13% coconut water. Average time required for production of single shoot and multiple shoot were 15-21 day and 40-45 day, respectively.

2.1.3 Shoot proliferation and differentiation of Banana

Demissie (2013) carried out an experiment on the effects of different combinations of BAP and NAA on multiple shoot proliferation of plantain (*Musa* sp.) cv. Matoke from meristem derived explant. The culture meristem grew into callus after 28 days then adventitious plantlets were developed. Among the different concentrations, 5.0 mg/L BAP + 1.0 mg/L NAA showed highest shoot proliferation (1.00, 1.67, 1.75 and 3.08 shoots per clump) at 10, 20, 30 and 60 DAI, respectively. Good numbers of shoots were achieved at 5.0 mg/L BAP + 0.5 mg/L NAA at 60 DAI (3.08). The longest shoot was produced at the concentration of 5.0 mg/L BAP + 1.0 mg/L NAA (0.43, 2.42, 2.63 and 3.42 shoots per plantlet) at 10, 20, 30 and 60 DAI, respectively. The maximum number of leaves at 10, 20, 30 and 60 DAI produced on the medium supplemented of with 5.0 mg/L BAP and 0.50 mg/L NAA are 1.67, 2.67, 3.67 and 4.33 per explants. The lowest number of leaves was obtained from the control treatment. The longest leaves were produced by the concentration of 5.0 mg/L BAP + 1.0 mg/L NAA (1.52, 2.27, 2.70 and 3.13 cm) at 10, 20, 30 and 60 DAI respectively.

Chariya (2012) studied on *Musa* sp. where series of media containing different concentration of BAP ranging from 1.0 to 6.0 mg/L. 1.0 mg/L BAP containing media, slow growth and 3-5 shoots of 2-3 cm in length was recorded. The higher numbers of shoots, 8-10, with 4-6 cm in length were observed with 5.0 mg/L BAP containing hard ball like structure developed from the meristem. The medium containing 6.0 mg/L BAP developed 6-8 shoots of 3-5 cm in length. Finally, 5.0 mg/L BAP concentration that gives higher number of shoots was chosen to carry out further culture process.

Ahirwar *et al.* (2012) observed the highest frequency of shoot regeneration of banana (*Musa paradisiaca* L.) (52.25%), number of shoots regenerated per explant (3.25) and shoot length (4.69 cm) from shoot tip explants cultured on MS medium supplemented with 5.0 mg/L BAP. Kinetin was found with highest frequency of shoot regeneration (44.56%), number of shoots regenerated per explant (2.75) and shoot length (3.5 cm) from shoot tip explants at concentration of 5.0 mg/L. The addition of 5.0 mg/LBAP was found better than kinetin for shoot development from shoot tip or male inflorescence tip explants. MS medium containing 7.5 mg/L BAP + 0.3 mg/L NAA

showed maximum shoot regeneration frequency than at other combination of BAP and NAA.

Al-amin *et al.* (2009) observed that among the different concentrations, 7.5 mg/L BAP + 0.5 mg/L NAA showed highest shoot proliferation of 0.75, 2.75 and 6.25 shoots per explant at 10, 20 and 30 DAI, respectively in BARI Banana-1. The longest shoot (1.03, 2.45 and 3.38 cm) at 10, 20 and 30 DAI, respectively, was produced by the treatment combination of 7.5 mg/L BAP + 0.5 mg/L NAA. The maximum numbers of leaves (2.50, 3.25 and 7.00 leaves/explant at 10, 20 and 30 DAI) were produced on the medium supplemented with the same treatment and it also produced the longest leaves, 0.85, 2.70 and 4.23 cm at 10, 20 and 30 DAI, respectively.

Sadik *et al.* (2007) cultured shoots of Banana cultivars on a multiplication media modified by adding a combination of BAP and TDZ for scalp generation. In their study the mean multiple bud proliferation increased as the concentration of TDZ increased in combination with BAP, they stated that higher multiple bud proliferation, which indicates better scalp formation was achieved in the treatments with TDZ than BAP.

Gubbuk and Pekmezc (2004) carried out an experiment on the *in vitro* propagation of some new banana types (*Musa* sp.). BAP (5, 10, 20 and 30 µM) and TDZ (0.4, 1, 2 and 3 µM) were tested alone and with 1 µM IAA for the propagation stage. Shoot proliferation and elongation were significantly greater with TDZ than with BAP in all 3 types. Furthermore, each cytokinin with IAA increased shoot proliferation and elongation more than their use alone. BAP below 20 µM or TDZ below 1 µM did not increase shoot proliferation, and BAP over 20 µM and TDZ over 2 µM suppressed shoot elongation.

2.1.4 Establishment of *in vitro* Banana plants in rooting media

In tissue culture, rooting of Banana plantlets is very important. A separate root induction phase is essential for rooting of Banana shoots before transferring them into soil. Various auxins at different concentrations are capable of root induction in micropropagated banana plantlets.

Fernandez *et al.* 2014 carried out an experiment on the potentiality of vermicompost humic acids in Banana *in vitro* micropropagation clone: *Enano Guantanamero*. The multiplication medium contained thiamine (2.0 mg/L), myo-inositol (100 mg/L), and sugar (30 g/L). By the use of HA, five treatments were used with total substitution of auxin and cytokinin (T1: HA (10 mg/L); (T2: HA (20 mg/L); (T3: HA (30 mg/L); (T4: HA (40 mg/L); (T5: HA (50 mg/L) and the control with 6 BAP (4.0 mg/L) + IBA (0.65 mg/L). The results showed that in the explants under HA treatments, elongation and cell multiplication were favored confirmed by explants great height, root number and length due to dry mass. The use of vermicomposting HA in the *in vitro* micropropagation of banana allow the elimination of rooting phase saving materials and be able to pass explants directly to acclimatization phase.

Iqbal *et al.* (2013) observed that for the proliferated shoots of banana (*Musa sapientum* L) transferred to different root induction media, which resultantly showed that MS media supplemented with IAA (2.0 mg/L), was the most efficient root inducing media. Rooted plantlets after primary and secondary hardening were transferred to the green house. Finally, these disease free plants were successfully established in soil.

Rahman *et al.* (2013) studied on IBA, NAA for induction of root of Banana cv. Agnishwar. IBA at a concentration of 1.0 mg/L was found most suitable for rooting of shoot.

Chariya (2012) conducted an experiment on *Musa* sp. using rooting medium containing different hormonal concentration ranging from 2.0 to 4.0 mg/L NAA and activated charcoal. After 10 days of transfer, 2.0 and 2.5 mg/L NAA containing medium had shown poor rooting. The rooting medium containing 3.0 mg/L NAA shown healthy growth of rooting with good quality numbers. With increase in hormonal concentration 3.5 and 4.0 mg/L NAA shoots were healthy but poor in numbers of root was observed. Thus, 3.0 mg/L NAA was considered better for root induction.

Ahirwar *et al.* (2012) observed that half strength MS medium containing 1.0 mg/L NAA was found suitable for root regeneration from shoots of Banana (*Musa paradisiaca* L.). In a study of *in vitro* plant regeneration in banana (*Musa* sp.) cv.

sabri by using meristematic stem cuttings explant, best response towards root induction was achieved on half MS medium supplemented with 0.5 mg/L IBA (Huq *et al.*, 2012).

Azam *et al.* (2010) observed that the micro shoots of Banana (*Musa* sp.) cultivar BARI-1 rooted well within two weeks in 1/2 MS supplemented with 0.5 mg/L IBA and in a few cases auto-root induction was observed when the number of subculture was beyond five. Rooting percentage and their growth were much better in liquid media in comparison to semi-solid media. Elimination of agar from the root induction media reduced the cost of production significantly.

Gitonga *et al.* (2010) reported that shoots of local Banana were rooted when they were transferred to MS medium supplemented with 1.0 mg /L NAA or 1.0 mg /L Anatone.

Karim *et al.* (2009) observed that regenerated shoots of Banana cv. BARI Banana-I were rooted on half strength MS medium containing 0.5 mg/L IAA + 0.5 mg/L IBA at 30 DAI.

Al-amin *et al.* (2009) investigated the effect of different concentrations of IBA and IAA on *in vitro* root formation of Banana cv. BARI Banana-I using half strength MS medium supplemented with different levels of IBA (0, 0.5, 0.1 and 1.5 mg/L) and IAA (0, 0.5 and 1.0 mg/L) was used. Root numbers varied with different concentrations of IBA and IAA. The highest numbers of roots were produced by 0.5 mg/L IAA + 0.5 mg/L IBA. The highest length (2.93, 4.63 and 5.88 cm) was recorded at 10, 20 and 30 DAI in the same treatment which was statistically significant.

High auxin in combination with low cytokinin induces root formation (Mala *et al.* 2005). Gubbuk and Pekmezc (2004) studied that charcoal alone was better for rooting than auxin treatments or MS medium alone. In conclusion, supplementation of 2.0 µM TDZ, and 1.0 µM IAA or 20.0 µM BAP and 1.0 µM IAA on MS medium, followed 5.0 g/L charcoal at the rooting stage were the best combinations for the *in vitro* propagation of banana types.

Muhammad *et al.* (2000, 2004) and Habiba *et al.* (2002) reported rooting of shoots cultured on half strength MS Medium having 1.0 and 2.0 mg/L IBA respectively and they found that 2.0 mg/L IBA was the best for root induction in the regenerated shoots.

Madhulatha *et al.* (2004, 2006) used IBA and NAA in combination during optimization of liquid pulse treatment for production of *in vitro* rooted plants of cv. Nendran (*Musa* sp. AAA).

An *in vitro* production of tetraploid Banana plantlets (*Musa* sp. FHIAOJ AAA group) was developed and the cloning effect on the *in vitro* development of the explants, the rate of contamination, multiplication and BAP concentrations were studied by Oliveira *et al.* (2001). They showed that higher multiplication rates were obtained, averaging 2.65 per sub-culture, on the MS media supplemented with 4.0 mg/L BAP. A pronounced effect of the cloning on multiplication was observed. Somaclonal variations were not observed among *in vitro* plantlets.

Molla *et al.* (2004) reported shoot tip of BARI Banana 1 were culture on MS medium supplemented with 5.0 mg/L BAP for shoot proliferation. Well-developed shoots were used for rooting. Among the six different concentrations of IBA (0.1, 0.2, 0.4, 0.5 and 0.6 mg/L) in half strength MS media, a good number of healthy roots were produced on 0.5 mg/L IBA (6.89) and 0.4 mg/L (6.31).

2.4 *Ex vitro* survival of plantlets

The survival rates of *in vitro* grown plantlets depend on the successful establishment of those under *ex vitro* conditions -

Rahman *et al.* (2013) reported that the rooted shoots of banana cv. Agnishwar were acclimatized and successfully transferred to plastic pots. After hardening, they were transferred to the main field and the survival rate was around 90%. This protocol might be used for the massive *in vitro* production of the plantlets of banana cv. Agnishwar.

Elisama *et al.* (2013) acclimatized of micropropagated *Musa cavendischii* cultivar roatan plants submitted to doses of fertigation and auxin. The experimental unit was one plant transplanted in each pot, resulting from the combinations of daily applications of 10 ml of the Steiner's nutritive solution at 10, 25, 50, 75 and 100%, respectively (Factor A), without and with 1.0 mg/L of the auxin IBA (Factor B). After 11 weeks of acclimatization, the results show that, the higher plants with respect to plant fresh, dry weight and height, and leaf width corresponded to the treatments from 75 to 100% of the Steiner's solution. The IBA application had no significant effects on the growth of the *M. cavendischii* plants. There was no significant interaction between fertigation and IBA applications.

Azam *et al.* (2010) reported that after proper acclimatization, rooted plantlets of Banana were transferred to polythene bags containing garden soil and humus (1:1). Two weeks after transplantation, 98% plants survived and flushed new leaves. No morphological variants were observed during the passage of micro-propagation.

Chariya (2012) observed that hardening the plantlets of *Musa spp.* was prepared with removing of agar media and then planted in individual cup containing sand: soil: wormiculite (1:1:1). Rooted plantlets were established for maintenance of humidity and temperature in a small chamber. A survival rate of 40-45% was achieved during the hardening.

Scaranari *et al.* (2009) carried out an experiment on the shading and periods of acclimatization of micropropagated Banana plantlets cv. Grande Naine. In this study, the development of Banana plantlets was evaluated during acclimatization under a full light condition including covered surfaces with red shade cloth (70%, 50%, and 30% shade) and black shade cloth (50% shade), both under a transparent plastic film of 100 mm. Temperature, relative air humidity, irrigation, and nutrition conditions were also controlled. Physical and physiological parameters were recorded at various stages in the greenhouses. Combined results indicated superior outcomes of plantlets maintained under black 50% shade cloth for nine weeks, both in the summer and winter seasons. Similar results, but in a shorter time, were obtained with plantlets cultivated under red 70% shade cloth, for six weeks in the summer.

Al-amin *et al.* (2009) reported that meristem derived plantlets were transferred to poly bags containing 1:1 (ground soil : cowdung) mixture after 7 days hardening in room temperature (28-30°C) and established plantlet of banana cv. BARI Banana-I was ready for planting.

Coasta *et al.* (2009) studied the physiological and anatomic performance, and *ex vitro* survival of micropropagated Banana plants in response to cultivation conditions, in the stage of *in vitro* rooting. Shoots of the 'Caipira' cultivar were cultivated in MS medium, supplemented with 1.0 mg/L NAA and 6.0 g/L agar, in which the following treatments were applied: two sucrose concentrations (15.0 g/L or 30.0 g/L) and two cultivation conditions (Natural light – greenhouse and Artificial light – growth chamber). At the end of 45 days, the contents of chlorophyll a, b and total, the relative water content in the tissues, anatomic characteristics and the *ex vitro* survival were evaluated. Effects of growth environment and sucrose concentration were observed on micropropagated 'Caipira' Banana anatomy, physiology and survival. *In vitro* rooting of the shoots under natural light in the medium containing 15 g/L or 30 g/L sucrose promoted major alteration in the increase of palisade and spongy parenchyma, as well as reducing leaf water loss and plant death. The results obtained in the present study confirm the potential of the use of natural light as a substitute for artificial light for micropropagation of tropical species.

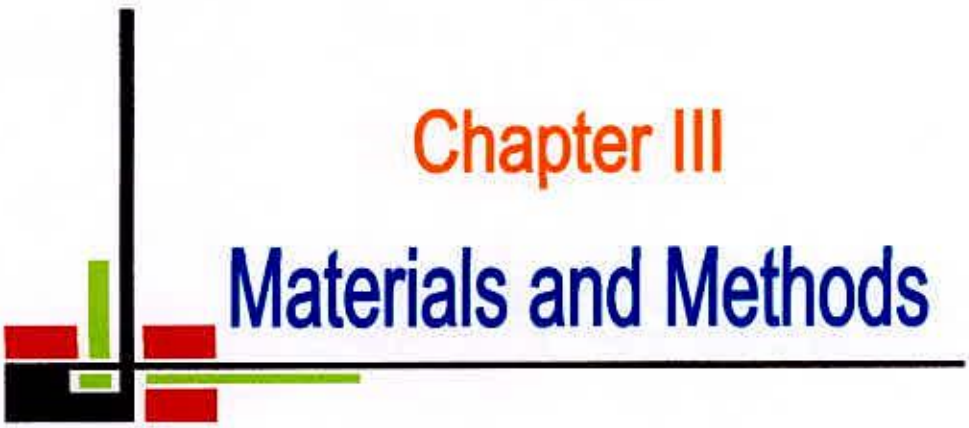
Kalimuthu *et al.* (2007) had also transferred the elongated shoots of *Musa* sp. with roots (about 8-9 cm) to hardening and achieved a survival rate of 90-95% during the process. Transplantation of *in vitro* derived banana plants to soil is often characterized by lower survival rates. Before transfer of soil rooted plants to their final environment, they must be acclimatized in a controlled environment room or in the glasshouse (Rohr *et al.* 2003).

Banana plantlet (*Musa* sp.) acclimatization can be divided into two phases. In the first, *in vitro* plantlets are transferred to controlled environments (green-house or box shade, under the conditions of 20 to 28°C, 80 to 90% RH, and 70% shade cloth) for a three to six-week period. In the second phase, plantlets are shifted to trays, pots or bags, under 50% shade, in a temperature range from 18°C to 34°C, and a relative humidity higher than 75%, for a gradual hardening (Souza *et al.*, 1997). When

plantlets reach 25–30 cm height they are considered acclimated and become available to the market (Silva *et al,*. 1999).

Molla *et al.* (2004) reported that plantlets transferred to plastic pots after 10, 15, 20, 25 and 30 days *in vitro* culture survived 95-100% plantlets of BARI banana 1 when they were transferred after 15-20 days *in vitro* culture with 7 days hardening at room temperature.

From the above review, it was evident that different plant growth regulators (PGRs) at different concentrations individually or in combinations influence *in vitro* culture of Banana varieties. However, very few investigations have yet been carried out on these factors.



Chapter III

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

3.1 Time and Location of the experiment

The present study was planned during October, 2013 to August, 2014 at the Biotechnology Laboratory, Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh. Materials used and methods followed to conduct the present study have been presented in this chapter.

3.2 Experimental materials

3.2.1 Plant materials

Two Banana (*Musa* spp.) varieties, Sagar and Sabri were used as experimental materials in the present investigation.

3.2.2 Source of materials

The planting materials of Banana (*Musa* spp.) Sagar and Sabri were collected from Agargau Nursery, Sher-e-Bangla Nager, Dhaka-1207.

3.2.3 Types of explants

The healthy, disease free shoot tips of 1-2 cm length were used as explants for the study for *in vitro* regeneration.

3.2.4 Culture media

The degree of success in tissue culture is related to the choice of nutritional components and growth regulators. MS (Murashige and Skoog, 1962) medium supplemented with different phytohormones as per treatments were used as culture medium for shoot induction, shoot multiplication and maintenance, and regeneration of roots from multiplied shoots. Hormones were added separately to different media according to the requirements. And for that stock solutions of hormones were prepared ahead of media preparation and stored at 4 °C temperature.

Table 1. Composition of Duchefa Biochemic MS (Murashige and Skoog, 1962) medium including vitamins

Components	Concentrations (mg/L)	Concentrations
Micro Elements	**mg/L**	**µM**
$CoCl_2.6H_2O$	0.025	0.11
$CuSO_4.5H_2O$	0.025	0.10
Fe Na EDTA	36.70	100.00
H_3BO_3	6.20	100.27
KI	0.83	5.00
$MnSO_4.H_2O$	16.90	100.00
$Na_2MoO_4.2H_2O$	0.25	1.03
$ZnSO_4.7H_2O$	8.60	29.91
Macro Elements	**mg/L**	**mM**
$CaCl_2$	332.02	2.99
KH_2PO_4	170.00	1.25
KNO_3	1900.00	18.79
$MgSO_4$	180.54	1.50
NH_4NO_3	1650.00	20.61
Vitamins	**mg/L**	**µM**
Glycine	2.00	26.64
Myo-Inositol	100.00	554.94
Nicotinic acid	0.50	4.06
Pyridoxine HCl	0.50	2.43
Thiamine HCl	0.10	0.30

Total concentration of Micro and Macro elements including vitamins: 4405.19 mg/L

Manufacturing Company: Duchefa Biochemic

3.3 Preparation of hormonal stock solutions

The first step in the preparation of the medium was the preparation of hormone stock solutions. To expedite the preparation of the medium separate stock solutions for growth regulators were prepared and used.

Separate stock solution of hormones was prepared by dissolving the desired quantity of ingredients to the appropriate solvent and made the final volume with distilled water and stored in a refrigerator at 4^0C for later use.

The following growth regulators and concentrations were used in this present investigation.

Auxins

Indole butyric acid (IBA) (0.5, 1.0, 1.5, 2.0 and 2.5 mg/L)

Cytokinins

6-benzyl amino purine (BAP) (1.0, 2.0, 3.0, 4.0, and 5.0 mg/L)

These hormonal supplements were dissolved in proper solvent as shown against each of them.

Hormones (Solute)	**Solvent**
IBA	70% ethyl alcohol
BAP	0.1 (N) NaOH

To prepare the stock solution of hormones (1 mg/ml), 100 mg of solid hormone was placed in a small beaker and then dissolved in 10 ml of 70% ethyl alcohol and 0.1 (N) NaOH solvent. Finally the volume was made upto 100 ml by the addition of sterile distilled water using a measuring cylinder. The prepared hormone solution was then labeled and stored at 4 ± 1^0C for use upto two month.

3.4 MS media preparation

After the preparation of the stock solution, the later step was the preparation of culture media. To prepare one liter of above mentioned media the following steps were followed:

i. 500 ml of sterile distilled water was added into 2 liter beaker.

ii. 4405.19 mg/L of Duchefa Biochemic MS medium including vitamins was added to beaker.

iii. Then thirty gram of sucrose was added and gently agitated to dissolve completely.

iv. Different concentrations of hormonal supplements were added to the solution either in single or in combinations as required and mixed well.

v. Since each hormonal stock contained 10 mg of the chemical in 100 ml of solution to make one litre of medium addition of 10 ml of stock solution of any of the hormones, resulted in 1 mg/L concentration of that hormonal supplement.

vi. The volume was made up to 1000 ml with addition of sterile distilled water.

vii. The pH of the medium was adjusted to 5.8 with a digital pH meter with the help of 0.1N HCl or 0.1N NaOH as necessary.

viii. After adjusting the pH, 8 g/L agar was added to solidify the medium.

ix. As additives, 1% of Activated charcoal was directly added to the medium.

x. The mixture was then heated for 10 minutes in an electric oven for melting of agar.

xi. Required volume of hot medium was dispensed into culture vessels *viz.*, vial. After dispensing the medium, the culture vessels were plugged with its cover and marked with different codes with the help of a glass marker to indicate specific media.

3.5 Sterilization

For *in vitro* techniques, aseptic condition is a prerequisite. So, all instruments, glassware and culture media were sterilized.

3.5.1 Sterilization of culture medium

The culture vessels containing the medium were autoclaved with 1.06 kg/cm^2 (15 PSI) of pressure at 121^{0}C for 20 minutes. After autoclaving the culture vessels (vials) containing the medium were allowed to cool in culture racks.

3.5.2 Sterilization of glassware and instruments

Beakers, test tubes, conical flasks, pipettes, metal instruments *viz.*, forceps, scalpels, needless, spatulas and aluminum foils were sterilized in an autoclave at a temperature of 121^{0}C for 20 minutes at 1.06 kg/cm^2 (15 PSI) pressure.

3.5.3 Sterilization of culture room and transfer area

The culture room was initially cleaned by gently washing all over the floors and walls with detergent or Lysol (germicide) followed by wiping with 70% ethyl alcohol. The process of sterilization was repeated at regular intervals. Generally, switching on the laminar airflow cabinet and sterilized the cabinet by wiping the working surface with 70% ethyl alcohol and then UV light was on for 30 minutes so that the working area of the cabinet is sterilized. After in the cabinet was delayed for at least 5 minutes to ensure safe environment.

3.6 Precaution to ensure aseptic condition

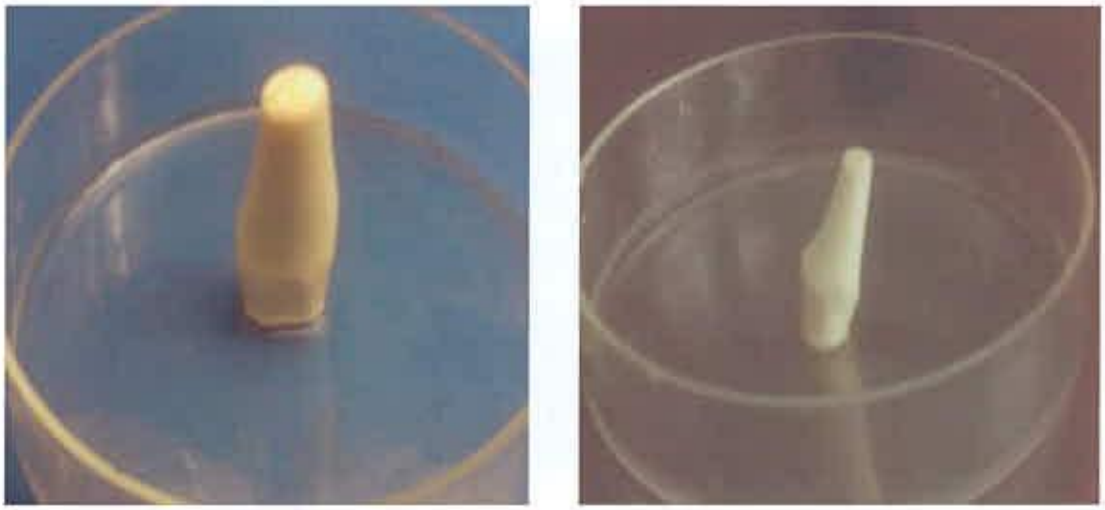
The cabinet was usually started half an hour before use and wiped with 70% ethyl-alcohol to reduce the chances of contamination. The instruments like forceps, scalpels, needles etc. were pre-sterilized by autoclaving and subsequent sterilization was done by dipping in 70% ethyl-alcohol followed by flaming and cooling. Hands were also sterilized by wiping with 70% ethyl-alcohol. Aseptic conditions were followed during each and every operation to avoid the contamination of culture.

3.7 Explant preparation and culture

3.7.1.1 Preparation of explant

Suckers (about three months of age) of Banana (Sagar and Sabri) grown under field conditions were used for explant preparation. The suckers were washed thoroughly under running tap water. The roots and outer tissues of the suckers were removed with the help of a sharp knife. A number of outer leaves were removed until the shoot measured about 1 to 2 cm length and 1 cm width at the base.

Plate 1. Banana plantlets of 2-3 months aged collected from nursery.



Sagar **Sabri**

Plate 2. Banana explants (shoot tips) prepared for placement in MS media.

3.7.1.2 Surface sterilization of explants

The shoots tips of 3 to 4 cm size were taken in a beaker and surface sterilization of explants was done as follows:

i. The suckers were cut as small size (3 to 4 cm) and rinsed with running tap water.
ii. The shoot tips were soaked with 5% Tween-20 solution for 10 min.
iii. Washing with distilled water was done for several times.
iv. One layer of shoot tip was removed and it was put into Bavistin solution (0.5%) that was supplemented with 100 mg/L Ascorbic acid and 150 mg/L Citric acid and kept for 20 min.
v. Rinsing the shoot tips with sterilized distilled water for at least 3 times.

Rests of activities were done in Laminar air flow cabinet.

vi. The shoot tips having 3-4 cm in length were isolated from initial stock.
vii. The shoot tips were sterilized with 70% ethanol for 1 min.
viii. Then the explants were sterilized with 0.2% $HgCl_2$ for 2 min.
ix. The explants were rinsed with sterilized distilled water for at least 3 times.
x. Outer layer of explants were removed carefully.
xi. Again the explants were sterilized with 0.1% $HgCl_2$ for 5 min.
xii. The explants were washed for at least 3 times with distilled water.
xiii. Another outer layer of the explants were removed carefully.
xiv. The final size of explants were 1-2 cm which had 6/8 overlapping leaf base enclosing auxiliary bud.
xv. Finally the explants were transferred to the MS media carefully.

3.7.2 Culture of explant

3.7.2.1 Inoculation of culture

The isolated and surface sterilized shoot tips were collected carefully through maintaining aseptic condition inside the laminar air flow cabinet. The individual shoot tips were directly inoculated to each of the culture tube containing 50 mL of MS medium supplemented with different concentrations of hormones as per treatment.

3.7.2.2 Incubation

The culture vials transferred to culture racks and allowed to grow in controlled environment. The temperature of the culture room was maintained within 25±1° C by an air conditioner and 16 hour photoperiod was maintained along with light intensity of 3000 lux for proper growth and development of culture.

3.7.2.3 Blackening of the explant

Some explant became black in color within 6-7 days after inoculation. To control blackening the blackish tissues on the explants were removed and the explants were transferred to similar fresh medium. It was repeated each of 10 days interval for about one month to minimize further blackening of the tissues.

3.7.2.4 Maintenance of proliferating shoots

Initial subculturing was done after 30 days when the explant had produced some shoots. For subculturing, the entire samples of *in vitro* shoot were cut into small pieces so that each piece would contain about one shoot. Leaf and blackish or brownish basal tissues were removed. Each piece was inoculated into a similar fresh medium. It was practiced at the interval of 20-25 days.

3.7.2.5 Regeneration of plants from *in vitro* proliferated buds

In vitro proliferated micro shoots were separated and each of the micro shoot was placed on culture medium, which was supplemented with particular concentration of hormone for shoot differentiation.

3.7.2.6 Root induction of regenerated shoots

When the shoots grew about 3-5 cm in length with 3-6 well developed leaves they were removed aseptically from the culture tubes and were separated from each other and again cultured on freshly prepared medium containing different combinations of hormonal supplements for root induction.

3.8 Hardening of the regenerated plantlets

Regenerated plantlets were transplanted to pots (10×15cm) containing soil and cowdung in 1:1 ratio and soil mixture were treated with a solution of 1% IBA. Occasional spray of water was done to prevent sudden desiccations and maintain high

humidity around the plantlets. Initially the plantlets were hardened in controlled environment. Then after 2 weeks, exposed to lower humidity and higher light intensity. Finally, after 20 days plantlets were transferred to natural environment.

3.9 Treatments

Three experiments were conducted to assess the effect of different concentrations of BAP and IBA on shoot proliferation and subsequent rooting of the multiplied shoot.

Experiment 1. Effect of BAP concentrations on multiple shoot proliferation from shoot tip explants in two Banana varieties

In this experiment, suckers of Sagar and Sabri varieties of Banana were used as sources of shoot tip to investigate the effect of BAP, at different concentration on shoot.

Treatments: Five levels of BAP (1.0, 2.0, 3.0, 4.0 and 5.0 mg/L) and a control (0.0 mg/L) were used.

Experiment 2. Effect of IBA concentrations on root formation of the micropropagated shoots in Sagar and Sabri varieties of Banana

IBA with five levels (0.5, 1.0, 1.5, 2.0 and 2.5 mg/L) and control (0.0 mg/L) were used as treatments.

Experiment 3. Combined effect of BAP and IBA on shoot and root regeneration in Sagar and Sabri varieties of Banana

Treatments: Combined hormone treatments with BAP in three levels as (3.0, 4.0 and 5.0 mg/L) and IBA with five levels as (0.5, 1.0, 1.5, 2.0 and 2.5 mg/L) were used for Sagar and Sabri varieties of Banana.

3.10 Data collection

Data were collected on the effect of different treatments on shoot and root proliferation on the following parameters recorded at 2, 4 and 6 weeks after inoculation (WAI).

3.10.1 Percent response of explants

Percent response of explants was calculated by using the following formula.

$$\text{Percent response of explants} = \frac{\text{Number of explant induced shoot}}{\text{Total number of explants inoculated}} \times 100$$

3.10.2 Days to shoot induction

Days to shoot induction were calculated by counting the days from explant inoculation to the first induction of shoots.

3.10.3 Number of shoots per explant

Number of shoots per explant was calculated by using the following formula,

$$\text{Number of shoots per explant} = \frac{\text{Number of shoots per explant}}{\text{Number of observation}}$$

3.10.4 Shoot length (cm)

Shoot length was measured in centimeter (cm) from the base to the top of the explants by a measuring scale. The mean was calculated.

3.10.5 Number of leaf

Numbers of leaves produced on the plantlet were counted and the mean was calculated.

3.10.6 Length of leaf (cm)

Leaf length was measured in centimeter (cm) from the base to the top of the leaves by a measuring scale. The mean was calculated.

3.10.7 Days to root formation

Days to root formation were calculated by counting the days from explant inoculation to the first induction of roots.

3.10.8 Number of roots

The number of roots per plantlet was counted and the mean was calculated.

3.10.9 Length of root (cm)

Root length was measured in centimeter from the base to the tip of the roots and the mean was calculated.

3.10.10 Percent increase over control (%)

The percent increase over control was calculated by using the following formula:

$$\text{Percent increase over control} = \frac{X_1 - X_2}{X_2} \times 100$$

Where, X_1 = the mean of treated explants,

X_2 = the mean of untreated explants

3.10.11 Percentage of established plantlets

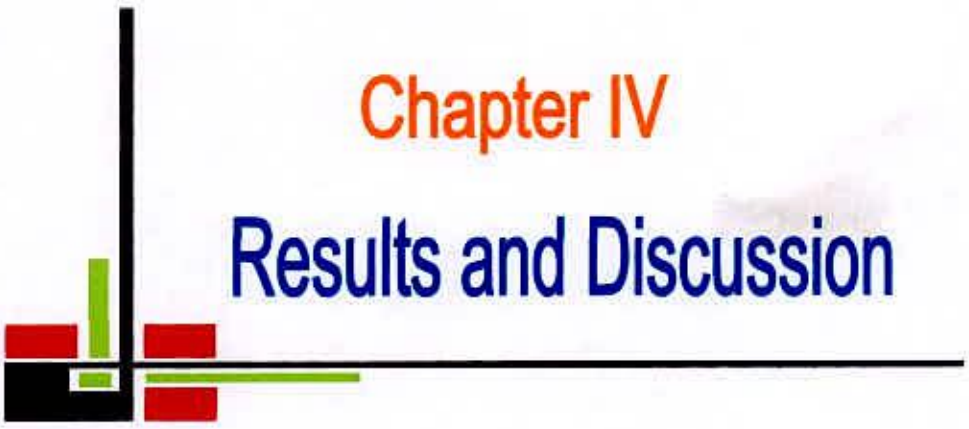
The percentages of established plantlets were calculated based on the number of plantlets placed in the plastic pots and the number of plants finally survived.

The percentages of established plantlet were calculated by using the following formula:

$$\text{Percentage of established plantlets} = \frac{\text{Number of established plantlets}}{\text{Total number of plantlets}} \times 100$$

3.11 Statistical analysis of data

The data for the characters under study were statistically analyzed wherever applicable. Data were analyzed using MSTAT-C statistical package. The experiment was conducted in culture room and arranged in Completely Randomized Design (CRD) single factor and two factors with five replications. The Analyses of Variance for different characters were performed and means were compared by the Duncan's Multiple Range Test (DMRT).



Chapter IV

Results and Discussion

CHAPTER IV

RESULTS AND DISCUSSION

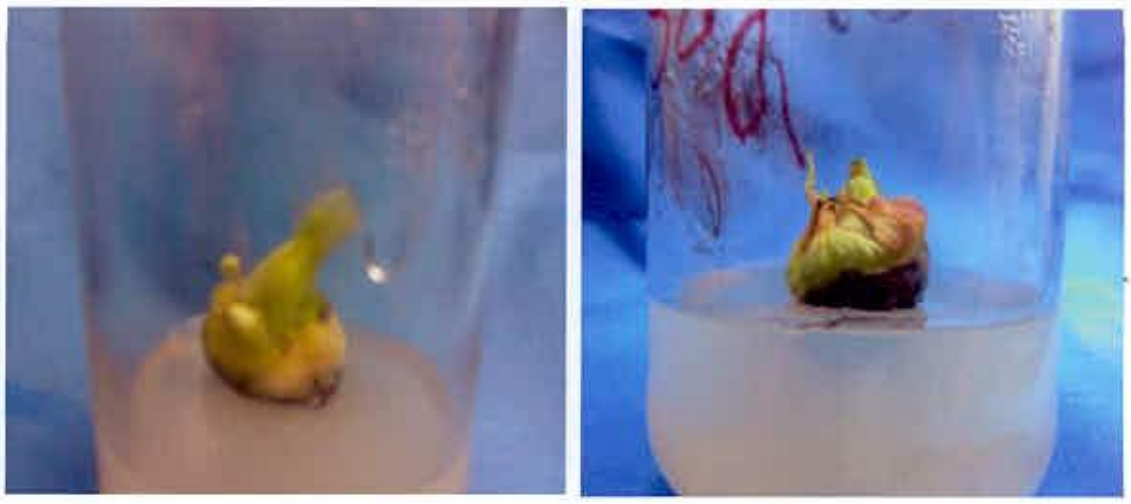
Regeneration of Banana plantlets through shoot tip culture offers the unique facilities of propagation with a view of obtaining planting materials free from diseases such as bunchy top and panama wilt. Thus the following works were conducted at the Biotechnology laboratory, Department of Biotechnology, Sher-e-Bangla Agricultural University with the Banana varieties of Sagar and Sabri using shoot tip as explant for subsequent shoot and root regeneration. The results of the experiment were presented and discussed in this chapter with Plates (1-22) and Tables (1-15). Analyses of variance in respect of all the parameters have been presented in Appendices I-LIV.

4.1 Experiment 1. Effect of BAP concentrations on multiple shoot proliferation from shoot tip explant in two varieties of banana

Plant regeneration and subsequent shoot and root multiplication from shoot tip of Sagar and Sabri varieties were done. MS medium supplemented with different concentrations of BAP was used and healthy shoot tips were collected and cut into pieces. Each piece having 1-2 cm long and about 1cm diameter was used as primary culture (explant) for shoot multiplication and proliferation. The results obtained from this experiment had been presented in Table 2-5 and discussed under following headings.

4.1.1 Regeneration of shoots from shoot tip explants

The effect of BAP on shoot proliferation and elongation from shoot tip of Sagar and Sabri varieties was investigated by adding different concentrations of BAP to a basal MS medium (semi solid). *In vitro* culture of shoot tip results green globular ball like structure within 7-10 days of inoculation in media containing different concentrations of BAP and combination of BAP and IBA (Plate 3). From these balls like structure adventitious plantlets were developing (Plate 4). Azam *et al.* (2010) found that cultured shoot tips were visible as a swelling and greenish color after 10–15 days of inoculation in MS media supplemented with different concentrations of BAP. Al-amin *et al.* (2009) observed meristematic ball like structure in regeneration media containing different concentrations of BAP and NAA.



Sagar **Sabri**

Plate 3. Green globular ball like structure produced from shoot tips of Sagar and Sabri varieties in MS media containing 4.0 mg/L and 5.0 mg/L of BAP, respectively at 8 days after inoculation.

Sagar **Sabri**

Plate 4. Adventitious shoot initiation from ball like structure of Sagar and Sabri varieties in MS media supplemented with 4.0 mg/L and 5.0 mg/L of BAP, respectively at 2 weeks after inoculation (WAI).

4.1.2 Percent response of explants (%)

Variations were observed among different treatments of benzyl amino purine (BAP) on percent response of explants (%) in Banana varieties cultured in MS media in the laboratory condition the results of which have been presented in the Figure 1. The highest percent response of explants (88%) of Sagar was observed in treatment of 4.0 mg/L BAP. The second highest (76%) was observed in treatment of 5.0 mg/L BAP and the lowest percent response of explants (40%) of Sagar was observed in control.

In case of Sabri, the highest percent response of explants (84%) was observed in 5.0 mg/L BAP (Table 2). The second highest (72%) was observed in 4.0 mg/L BAP while the lowest (44%) was observed in control (0.0 mg/L). Ahirwar *et al.* (2012) found the highest frequency of shoot regeneration (52.25%) at 5.0 mg/L BAP using Banana (*Musa paradisiaca* L.) that is supported by the present study. The findings of the present study is not fully supported by Darvari *et al.* (2010) where they found that all the cultivars of Malaysian Banana and plantain (*Musa* spp.) showed their highest response to regeneration at 8.0 mg/L of BAP.

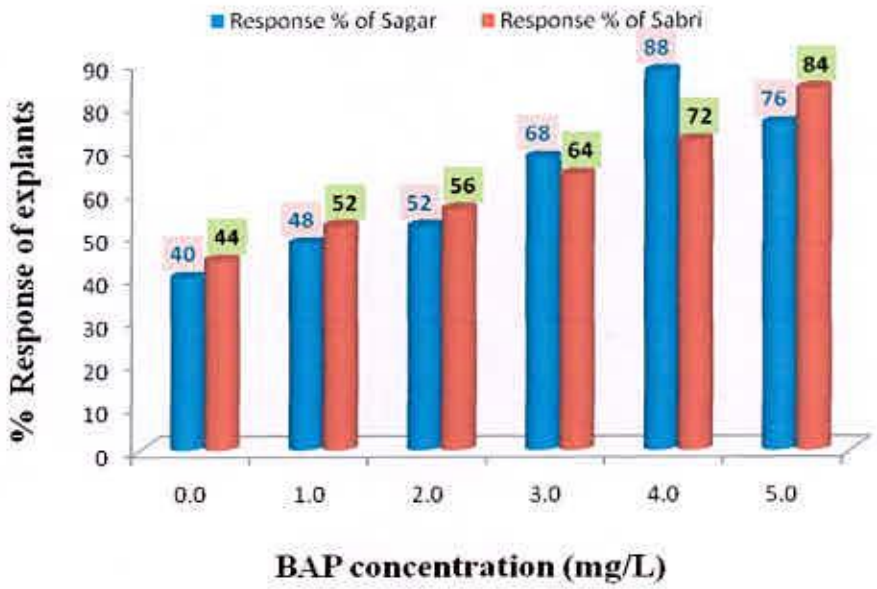


Figure 1. Effect of BAP on the percent response of explants in two varieties of Banana

4.1.3 Days to shoot induction

With different concentrations of BAP, significant variations were observed on days to shoot induction in Sagar and Sabri varieties of Banana. In both Sagar and Sabri, the highest number of 22.0 and 28.0 days, respectively was recorded for shoot induction in control (0.0 mg/L). The second highest number of days (11.40) was observed at 5.0 and 2.0 mg/L BAP and the minimum number of 7.80 days were required for shoot induction at 4.0 mg/L BAP in Sagar. For Sabri, the lowest number of days (9.40) had been recorded for shoot induction at 5.0 mg/L BAP (Table 2). Chariya (2012) observed the shoot initiation of *Musa* sp. after 12 days of inoculation in each media containing 1.0 to 6.0 mg/L BAP. This variation may be due to the strong growth potential of shoot tip and presence of sufficient endogenous cytokinin that support growth and development of shoot tip explant.

Table 2. Effect of BAP on days to shoot induction of two Banana varieties

BAP (mg/L)	Days to shoot induction	
	Sagar variety	Sabri variety
0.0	22.00±1.58a	28.00±1.58a
1.0	8.20 ±0.84c	13.20±1.30b
2.0	11.40±1.14b	11.80±1.48bc
3.0	9.00 ±1.00c	12.00±1.58b
4.0	7.80±0.84c	13.40±1.14b
5.0	11.40±1.14 b	9.40±1.14c
$LSD_{(0.01)}$	1.98	2.45
CV (%)	9.61	9.46
SE	0.50	0.62

Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT

4.1.4 Number of shoots per explant

Significant variations at 1% level were observed among different treatments of BAP on number of shoots per explant in Sagar variety of Banana using MS media (Figure 2 and Appendix I). At 2, 4 and 6 weeks after inoculation (WAI) the highest number of shoots observed in Sagar were 2.60, 4.60 and 6.80 per explant, respectively, in medium supplemented with 4.0 mg/L BAP. The second highest (2.20, 3.00 and 5.00 shoots per explant) was observed in 3.0 mg/L BAP at 2, 4 and 6 WAI, respectively. The 5.0 mg/L of BAP also showed good performance (1.40, 2.60 and 3.60 shoots per explant) over the control.

In the case of Sabri (Figure 3 and Appendix II), the highest number of shoots (2.00, 3.00 and 3.40 shoots per explant) was observed in medium supplemented with 5.0 mg/L BAP at 2, 4 and 6 WAI, respectively. The better performance was observed in 4.0 mg/L of BAP (3.00 shoots per explant) at 6 WAI. The 3.0 mg/L of BAP (1.60, 2.00 and 2.40 shoots per explant) also showed good performance. In both cases, the lowest number of shoot was observed in control (1.00, 1.00 and 1.00 shoots per explant). The result of current investigation is supported by Rahman *et al.* (2013) where they found the highest number of shoots (5.9) for each explant at 4.0 mg/L BAP using Banana (*Musa* sp.) cv. Agnishwar. Al-Amin *et al.* (2009) observed that if the explant of BARI Banana-1 in the culture media is not contaminated by fungus or bacteria then the explant develops only a single shoot in the long run. The high performance of BAP over other cytokinins in the multiplication of shoot tips has also been reported in different cultivar of banana by Gilmar *et al.* (2000). The initial response of cytokinin may be mediated by an increase in the cytosolic calcium concentration by promoting calcium uptake from the medium. Calcium affects the cytoskeleton, which can regulate exocytosis (Hager *et al.*, 1991)

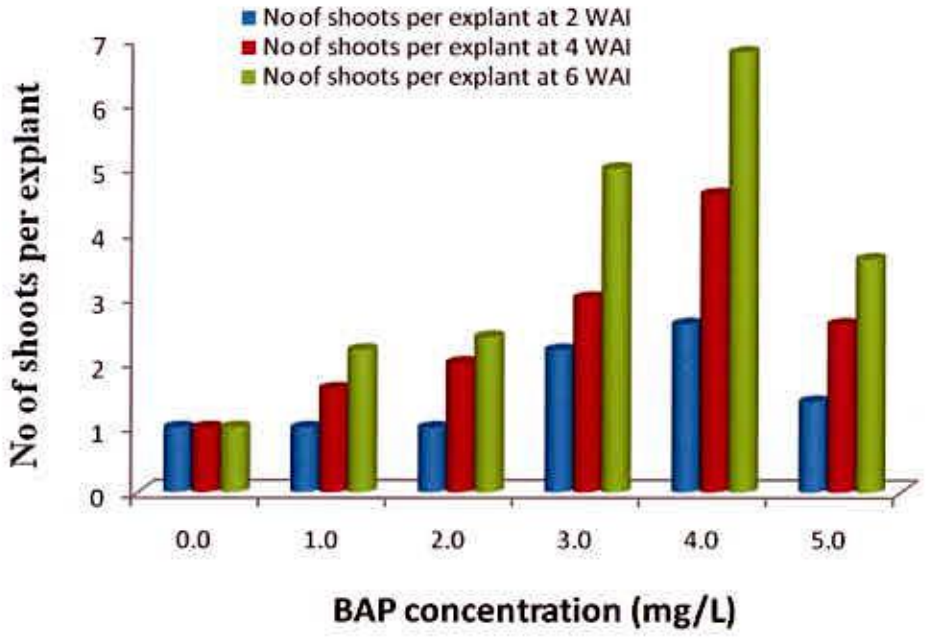


Figure 2. Effect of BAP on number of shoots per explant of "Sagar" variety.

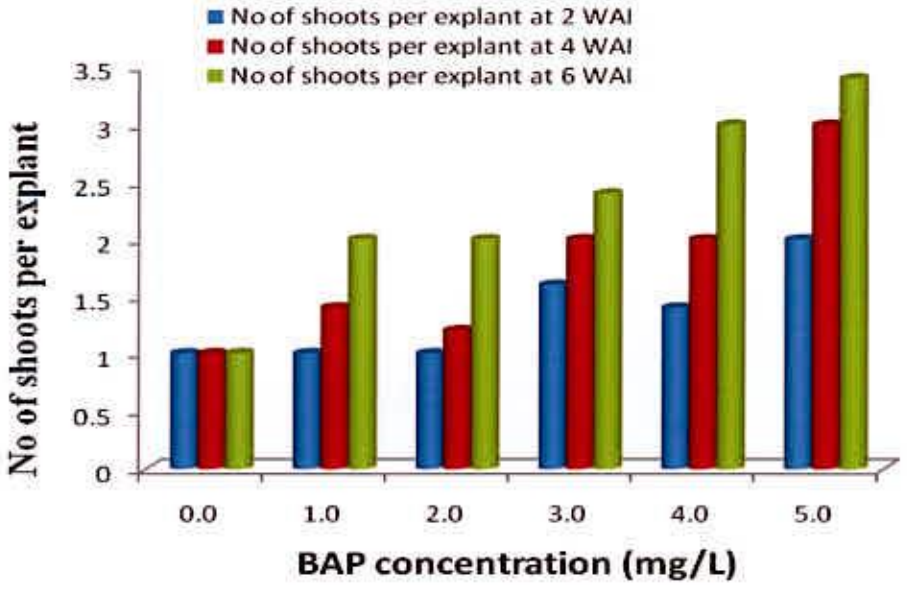


Figure 3. Effect of BAP on the number of shoots per explant of "Sabri" variety.

Plate 5. Multiple shoots produced from shoot tip of "Sagar" variety of Banana cultured on MS medium supplemented with 4.0 mg/L BAP at 6 WAI.

Plate 6. Multiple shoots produced from shoot tip of "Sabri" variety of Banana cultured on MS medium supplemented with 5.0 mg/L BAP at 6 WAI.

4.1.5 Length of shoot (cm)

Different treatments of BAP showed significant variations on length of shoot (cm) in Sagar variety of Banana at 1% level of significance (Table 3a). The highest length of shoot (3.69, 5.01 and 8.31 cm) was observed in treatment of 4.0 mg/L BAP at 2, 4 and 6 weeks after inoculation (WAI), respectively which was statistically similar with 3.0 mg/L (3.61 and 4.72 cm) at 2 and 4 WAI, respectively. On the other hand, the lowest length of shoot (1.18, 1.50 and 2.27 cm) was observed in control (0.0 mg/L) at 2, 4 and 6 WAI, respectively which was statistically different from all other treatments. Considering the per cent increase of shoot by length over control, the highest increase (212.71, 234.00 and 266.08%, respectively) was observed in 4.0 mg/L at 2, 4 and 6 WAI, respectively. On the other hand, minimum increase over control was observed in 1.0 mg/L (143.22, 150 and 143.17%, respectively) at 2, 4 and 6 WAI, respectively.

In case of Sabri (Table 3b), the highest length of shoot (3.22 and 4.09 cm) was observed in 4.0 mg/L which was statistically similar with 5.0 mg/L (3.01 and 3.98 cm) at 2 and 4 WAI, respectively and 4.0 mg/L (6.19 cm) that was statistically identical with 5.0 mg/L (6.07 cm) and 3.0 mg/L (5.99 cm) at 6 WAI, respectively. On the other hand, the lowest length of shoot (1.66, 1.77 and 2.30 cm) was observed in control (0.0 mg/L) at 2 and 4 WAI, respectively which was statistically different with all other treatments. Considering the per cent increase of shoot by length over control, the highest increase (93.98, 131.07 and 169.13%, respectively) was observed in 4.0 mg/L, followed by 5.0 mg/L (81.33, 124.86 and 163.91%, respectively) at 2, 4 and 6 WAI, respectively. On the other hand, minimum increase over control was observed in 2.0 mg/L (56.63, 87.01 and 132.61%, respectively) at 2, 4 and 6 WAI. Rahaman *et al.* (2004) obtained the longest shoots in 5.0 mg/L BAP (3.62 cm) followed by 1.5 mg/L NAA and 4.0 mg/L BAP (3.40 cm) using BARI Banana-1. They also found shortest shoot length (1.05 cm) in control treatment where growth hormones were absent.

Table 3a. Effect of BAP on the length of shoot of Sagar variety of Banana

BAP (mg/L)	Length of shoot (cm)					
	2 WAI*	% Increase over control	4 WAI*	% Increase over control	6 WAI*	% Increase over control
0.0	1.18±0.12e	-	1.50±0.17e	-	2.27±0.18e	-
1.0	2.87±0.09d	143.22	3.75±0.17d	150.00	5.52±0.10d	143.17
2.0	3.19±0.17cd	170.33	4.17±0.32cd	178.00	5.95±0.38cd	162.11
3.0	3.61±0.18ab	205.93	4.72±0.23ab	214.67	7.28 ±0.18b	220.70
4.0	3.69±0.26a	212.71	5.01±0.24a	234.00	8.31±0.20a	266.08
5.0	3.31±0.28bc	180.51	4.35±0.30bc	190.00	6.24 ±0.46c	174.89
$LSD_{(0.01)}$	0.34		0.43		0.47	
CV (%)	6.51		6.24		4.74	
SE	0.09		0.11		0.12	

*WAI=Weeks after inoculation. Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT.

Table 3b. Effect of BAP on the length of shoot of Sabri variety of Banana

BAP (mg/L)	Length of shoot (cm)					
	2 WAI*	% Increase over control	4 WAI*	% Increase over control	6 WAI*	% Increase over control
0.0	1.66 ±0.12d	-	1.77±0.15 d	-	2.30±0.45c	-
1.0	2.62±0.19c	57.83	3.41±0.15 c	92.66	5.38±0.23b	133.91
2.0	2.60 ±0.16c	56.63	3.31±0.10 c	87.01	5.35±0.14b	132.61
3.0	2.84±0.21bc	71.08	3.75 ±0.18b	111.86	5.99±0.29a	160.43
4.0	3.22 ±0.29a	93.98	4.09±0.21 a	131.07	6.19±0.18a	169.13
5.0	3.01±0.24ab	81.33	3.98±0.18ab	124.86	6.07±0.23a	163.91
$LSD_{(0.01)}$	0.33		0.29		0.48	
CV (%)	7.09		4.88		5.20	
SE	0.08		0.07		0.12	

*WAI=Weeks after inoculation. Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT.

Plate 7. Longest shoot produced from shoot tip in "Sagar" variety of Banana cultured on MS medium supplemented with 4.0 mg/L BAP at 4 WAI.

Plate 8. Longest shoot produced from shoot tip in "Sagar" variety of Banana cultured on MS medium supplemented with 4.0 mg/L BAP at 6 WAI.

Plate 9. Longest shoot produced from shoot tip of "Sabri" variety of Banana explant cultured on MS medium supplemented with 4.0 mg/L BAP at 4 WAI.

Plate 10. Longest shoot produced from shoot tip of "Sabri" variety of Banana explant cultured on MS medium supplemented with 4.0 mg/L BAP at 6 WAI.

4.1.6 Leaf number per explant

The significant variations were observed among different concentrations of BAP on number of leaf per explant (Table 4a). BAP of 4.0 mg/L showed highest number of leaf (3.01, 3.29 and 4.24) at 2, 4 and 6 WAI, respectively. The lowest number of leaf (0.60, 1.00 and 1.60) was observed in untreated control (0.0 mg/L) at 2, 4 and 6 WAI, respectively. Considering the per cent increase of number of leaf over control, 4.0 mg/L treatment showed the highest increase (401.67, 229.00 and 165.00%, respectively) at 2, 4 and 6 WAI, respectively. The minimum increase over control was observed in 1.0 mg/L (100.00, 70.00 and 83.13%, respectively) at 2, 4 and 6 WAI, respectively.

Sabri variety of Banana showed (Table 4b) highest number of leaf (2.54, 3.38 and 4.49) at 5.0 mg/L which was statistically similar with 4.0 mg/L (2.17, 2.87 and 4.17) at 2, 4 and 6 WAI, respectively. On the contrary, the untreated control showed the lowest average number of leaf (0.60, 1.00 and 1.80) at 2, 4 and 6 WAI, respectively. Considering the per cent increase of average number of leaf over control, the maximum increase (323.33, 238.00 and 149.44%, respectively) was observed in 5.0 mg/L BAP and the lowest increase (133.33 and 75.00%, respectively) over control was observed in 1.0 mg/L at 2 and 6 WAI, respectively. According to Chariya, (2012), 2-4 leaves with healthy development of shoot was observed in media containing 5.0 mg/L BAP, while with 6.0 mg/L BAP in media two leaves showing slight healthy shoots was observed in *Musa* sp.

Table 4a. Effect of BAP on the leaf number in Sagar variety of Banana

BAP (mg/L)	Leaf number per explants					
	2 WAI*	% Increase over control	4 WAI*	% Increase over control	6 WAI*	% Increase over control
0.0	0.60 ±0.55c	-	1.00 ±0.0d	-	1.60 ±0.55d	-
1.0	1.20±0.45bc	100.00	1.70 ±0.67c	70.00	2.93 ±0.38c	83.13
2.0	1.60 ±0.55b	166.67	2.47 ±0.29b	147.00	3.36±0.23bc	110.00
3.0	2.62 ±0.23a	336.67	3.01±0.22ab	201.00	3.55±0.36bc	121.88
4.0	3.01±0.22a	401.67	3.29 ±0.31a	229.00	4.24 ±0.24a	165.00
5.0	2.42±0.43a	303.33	2.67±0.24ab	167.00	3.59 ±0.12b	124.38
$LSD_{(0.01)}$	0.75		0.62		0.60	
CV (%)	22.28		14.87		10.66	
SE	0.19		0.16		0.15	

*WAI=Weeks after inoculation. Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT.

Table 4b. Effect of BAP on the leaf number in Sabri variety of Banana

BAP (mg/L)	Leaf number per explants					
	2 WAI*	% Increase over control	4 WAI*	% Increase over control	6 WAI*	% Increase over control
0.0	0.60 ±0.55c	-	1.00 ±0.0d	-	1.80± 0.45d	-
1.0	1.40±0.55bc	133.33	2.10±0.74bc	110.00	3.15±0.78c	75.00
2.0	1.60±0.55ab	166.67	1.90±0.55c	90.00	3.42±0.25bc	90.00
3.0	2.10±0.74ab	250.00	3.18±0.46a	218.00	3.99±0.43abc	121.67
4.0	2.17±0.21ab	261.67	2.87±0.22ab	187.00	4.17±0.43ab	131.67
5.0	2.54 ±0.30a	323.33	3.38±0.26a	238.00	4.49 ±0.30a	149.44
$LSD_{(0.01)}$	0.91		0.78		0.84	
CV (%)	29.64		18.42		13.48	
SE	0.23		0.20		0.21	

*WAI=Weeks after inoculation. Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT.

Plate 11. **Highest number of leaves produced from shoot tip in "Sagar" variety of Banana cultured on MS medium supplemented with 4.0 mg/L BAP at 6 WAI.**

Plate 12. **Highest number of leaves produced from shoot tip of "Sabri" variety of Banana cultured on MS medium supplemented with 5.0 mg/L BAP at 6 WAI.**

4.1.7 Length of leaf (cm)

The effect of different concentrations of BAP on the length of leaf (cm) of Sagar variety of Banana showed significant variations (Table 5a) at 1% level. The highest length of leaf (2.01, 2.77 and 4.25 cm) at 2, 4 and 6 WAI, respectively was observed in 3.0 mg/L BAP. This treatment effect was statistically similar with 4.0 mg/L (1.88 and 2.58 cm) at 2 and 4 WAI, respectively. The lowest length of leaf (0.50, 1.01 and 1.70 cm) was observed in untreated control at 2, 4 and 6 WAI, respectively, which was statistically different from all other treatments. Considering the per cent increase of leaf by length over control, at 2, 4 and 6 WAI, respectively, the highest increase (34.00, 174.26 and 150.00%, respectively) was observed in 3.0 mg/L BAP and the minimum increase over control was observed in 1.0 mg/L (6.67, 110.89 and 87.65%, respectively).

The highest length of leaf (2.35, 2.92 and 4.44 cm) in Sabri variety of Banana (Table 5b) was observed in 5.0 mg/L BAP and the lowest length of leaf (0.54, 0.99 and 1.78 cm at same interval) was observed in growth regulator free control at 2, 4 and 6 WAI, respectively, had been found statistically different from all other treatments. Considering the per cent increase of leaf by length over control, the highest increase (335.19, 194.95 and 149.44%, respectively) was observed in 5.0 mg/L BAP at 2, 4 and 6 WAI, respectively. On the other hand, lowest increase over control was observed in 1.0 mg/L (85.96 %) at 6 WAI. Al-amin *et al.* (2009) obtained the longest leaves, 0.85, 2.70 and 4.23 cm with 7.5 mg/L BAP + 0.5 mg/L NAA at 10, 20 and 30 DAI, respectively using BARI Banana-1. The different results obtained by different authors might be due to differences of genotypes and explants used.

Table 5a. Effect of BAP on the length of leaf in Sagar variety of Banana

BAP (mg/L)	Length of leaf (cm)					
	2 WAI*	% Increase over control	4 WAI*	% Increase over control	6 WAI*	% Increase over control
0.0	0.50±0.47b	-	1.01 ±0.33d	-	1.70±0.38d	-
1.0	1.60±0.09a	6.67	2.13 ±0.13c	110.89	3.19±0.14c	87.65
2.0	1.66±0.08a	10.67	2.31 ±0.21bc	128.71	3.26±0.19bc	91.76
3.0	2.01±0.14a	34.00	2.77 ±0.20a	174.26	4.25±0.17a	150.00
4.0	1.88±0.17a	25.33	2.58 ±0.17ab	155.45	3.66±0.21b	115.29
5.0	1.75±0.08a	16.67	2.47±0.23abc	144.55	3.47±0.16bc	104.12
$LSD_{(0.01)}$	0.39		0.39		0.39	
CV (%)	14.03		9.98		6.82	
SE	0.10		0.10		0.10	

*WAI=Weeks after inoculation. Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT.

Table 5b. Effect of BAP on length of leaf in Sabri variety of Banana

BAP (mg/L)	Length of leaf (cm)					
	2 WAI*	% Increase over control	4 WAI*	% Increase over control	6 WAI*	% Increase over control
0.0	0.54 ±0.50c	-	0.99 ±0.24c	-	1.78 ±0.0c	-
1.0	1.82 ±0.12b	237.04	2.13 ±0.14b	115.15	3.31±0.27b	85.96
2.0	1.75 ±0.11b	224.07	2.36±0.58ab	138.38	3.33±0.20b	87.08
3.0	1.93±0.12ab	257.41	2.51±0.44ab	153.54	3.98±0.40a	123.60
4.0	2.21±0.18ab	309.26	2.64±0.26ab	166.67	4.18±0.51a	134.83
5.0	2.35 ±0.22a	335.19	2.92 ±0.28a	194.95	4.44±0.33a	149.44
$LSD_{(0.01)}$	0.44		0.63		0.57	
CV (%)	14.05		15.68		9.25	
SE	0.11		0.16		0.15	

*WAI=Weeks after inoculation. Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT.

4.2 Experiment 2. Effect of IBA concentrations on root formation of the micropropagated shoots in Sagar and Sabri varieties of Banana.

The regenerated shoots were collected from *in vitro* grown plantlets in experiment 1. Then the shoots were subcultured in MS medium supplemented with different levels of IBA (0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L) in order to root formation. Roots numbers varied with different concentrations of IBA. The results on the effect of different concentrations of IBA on root formation had been discussed with following headings.

4.2.1 Days to root formation

Significant variations were observed at different concentrations of IBA on the number of days to root formation in Sagar and Sabri varieties of Banana (Table 6). In Sagar and Sabri, the highest number of days (15.20 and 14.20 days) were required for root formation was found in control followed by 2.5 mg/L IBA (9.80 and 10.80 days) and the lowest number of days (7.60 and 8.00 days) were required for root formation was recorded in 1.5 mg/L IBA in Sagar and Sabri varieties of Banana. Ali *et al.* (2011) found that MS medium supplemented with 1.0 mg/L IBA + 0.5 mg/L NAA showed 3.6 roots per planlet after 6.8 days of inoculation into rooting medium.

Table 6. Effect of IBA on days to root formation in two Banana varieties

IBA (mg/L)	Days to root formation	
	Sagar variety	Sabri variety
0.0	15.20±1.00a	14.20±1.00a
0.5	8.00 ±1.58b	9.00 ±1.58bc
1.0	8.00±1.58b	8.60 ±1.14c
1.5	7.60±1.14b	8.00 ±1.00c
2.0	8.00±1.58b	9.60 ±1.14bc
2.5	9.80±0.84b	10.80 ±0.084b
$LSD_{(0.01)}$	2.23	1.88
CV (%)	18.24	13.89
SE	0.56	0.48

Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT

4.2.2 Number of roots per plantlet

The significant variations were observed among different treatments of IBA on growth parameters of Sagar and Sabri variety of Banana such as number of root at 2, 4 and 6 WAI in MS media at 1% level of significance (Figure 4). The highest number of roots (2.00, 3.20 and 5.20 per plantlet) at 2, 4 and 6 WAI, respectively were developed by 0.5 mg/L of IBA for Sagar (Plate 13). On the other hand, the lowest number of roots (0.40 per plantlet) at 6 WAI were produced by the untreated control in both Sagar and Sabri (Plate 14). In case of Sabri, the highest number of root (2.20, 3.40 and 3.40 per plantlet) was produced by 1.5 mg/L of IBA, which was statistically similar with 1.0 mg/L IBA (2.20, 2.60 and 2.60 per plantlet) at 2, 4 and 6 WAI, respectively (Figure 4). Vigorous roots of *in vitro* grown plantlet of Sagar and Sabri varieties of Banana on MS media supplemented with 0.5 mg/L IBA and 1.5 mg/L IBA were shown in Plate 13. The results of present study agree with the findings of Rahman *et al.* (2013). They observed that IBA at a concentration of 1.0 mg/L was found most suitable for rooting of shoot in Banana cv. Agnishwar. Huq *et al.* (2012) found that the best response of Banana (*Musa* spp.) cv. Sabri towards root induction was achieved on half MS medium supplemented with 0.5 mg/L IBA. This variation in number of roots per plantlet might be due to the difference in genotype and culture environments.

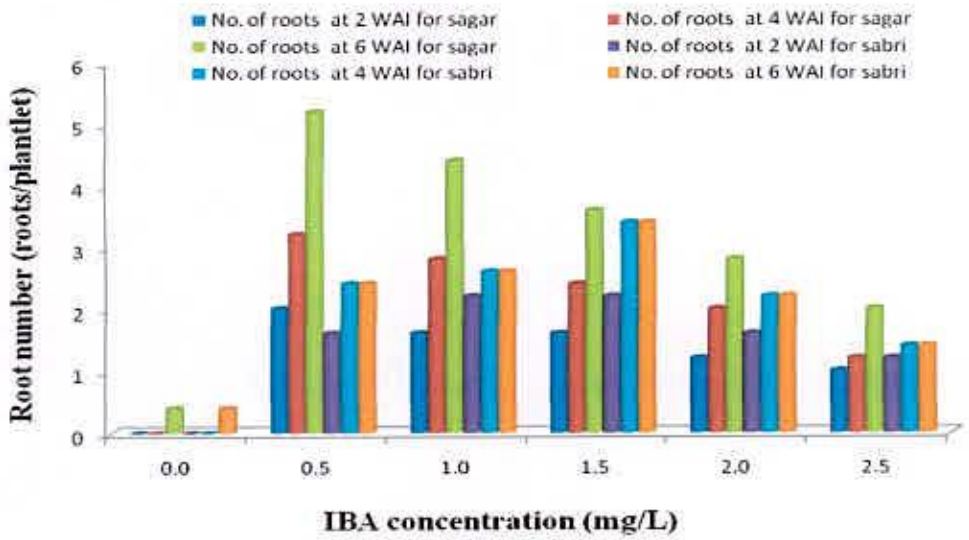


Figure 4. Effect of IBA on number of roots/plantlet for "Sagar" and "Sabri" variety of Banana.

Sagar **Sabri**

Plate 13. Vigorous roots of "Sagar" and "Sabri" varieties of Banana grown with 0.5 mg/L IBA and 1.5 mg/L IBA.

Sagar **Sabri**

Plate 14. Roots of "Sagar" and "Sabri" varieties of Banana grown with untreated control (0.0 mg/L).

4.2.3 Length of root (cm)

The significant variations were found among different combinations of IBA on the length of root (cm) of Sagar variety of Banana at 1% level of significance (Table 7). The highest length of root (2.23, 3.15 and 4.29 cm) was recorded in 0.5 mg/L IBA at 2, 4 and 6 WAI, respectively which was statistically similar with 1.0 mg/L IBA (2.09 and 2.93 cm) at 2 and 4 WAI, respectively. In Sabri variety of Banana (Table 7) the highest length of root (3.65 cm) was found in 0.5 mg/L IBA which was statistically identical with 1.0 mg/L IBA at 6 WAI. On the other hand, lowest length of root (0.20 cm) was produced in untreated control in both Sagar and Sabri varieties of Banana. Rahman *et al.* (2013) recorded that the root length ranged from 1.61 cm to 3.69 cm under 0.5, 1.0 and 2.0 mg/L of IBA.

Table 7. Effect of IBA on length of root for Sagar and Sabri varieties of Banana

IBA (mg/L)	Length of root (cm)					
	Sagar variety			Sabri variety		
	2 WAI*	4 WAI*	6 WAI*	2 WAI*	4 WAI*	6 WAI*
0.0	0.0 ±0.0d	0.0 ±0.0c	0.20 ±0.0 e	0.0 ±0.0c	0.0 ±0.0d	0.20±0.0c
0.5	2.23±0.15a	3.15±0.11a	4.29±0.26a	1.74 ±0.10a	2.40 ±0.08a	3.65±0.26a
1.0	2.09±0.17a	2.93±0.20a	3.96±0.23b	1.66±0.12ab	2.38 ±0.12a	3.65±0.23a
1.5	1.84±0.15b	2.54±0.27b	3.55±0.32c	1.59±0.12ab	2.33±0.12ab	3.42±0.32ab
2.0	1.58±0.11c	2.30±0.22b	3.29±0.17cd	1.53 ±0.10b	2.18±0.13bc	3.16±0.17b
2.5	1.48±0.13c	2.20±0.23b	3.12±0.20d	1.48 ±0.08b	2.08 ±0.08c	3.15±0.20b
$LSD_{(0.01)}$	0.23	0.35	0.32	0.17	0.17	0.40
CV (%)	8.57	8.99	5.97	7.01	5.06	7.95
SE	0.60	0.09	0.08	0.04	0.04	0.10

*WAI = Weeks after inoculation. Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT.

4.3 Experiment 3. Combined effect of BAP and IBA on shoot and root regeneration in Sagar and Sabri varieties of Banana

In previous experiment the effect of BAP at different levels reveals better performance of Sagar and Sabri varieties at higher BAP concentrations. That is why in this experiment the effect of 3.0, 4.0 and 5.0 mg/L BAP in Sagar and Sabri varieties of Banana was investigated under following headings.

4.3.1 Percent response of explants (%)

The different concentrations of BAP and IBA showed significant variations on percent response of explants (%) in Sagar variety of Banana (Table 8). The highest percent response of explants (85%) was observed in combination of 4.0 mg/L BAP and 2.0 mg/L IBA and the lowest percent response of explants (35%) was observed in treatment combination of 3.0 mg/L BAP +2.5 mg/L IBA.

Sabri variety of Banana showed (Table 8) the highest percent response of explants (90%) at 5.0 mg/L BAP + 2.5 mg/L IBA and the lowest response (35%) at 3.0 mg/L BAP +2.0 mg/L IBA. The results of present study are partially supported by Huq *et al.* (2012) where they found the highest percentage of shoot regeneration (90%) when cultured on MS + 4.0 mg/L BAP + 2.0 mg/L IAA + 13% (v/v) coconut water using Banana (*Musa* spp.) cv. Sabri. According to Cronauer and Krikorian (1984a), the requirement of cytokinin and auxins depends on the variety of Banana and culture conditions.

Table 8. Combined effect of BAP and IBA on percent response of explants in Sagar and Sabri varieties of Banana

BAP (mg/L)	Number of explant inoculated	IBA (mg/L)	Percent response of explants (%)	
			Sagar variety	Sabri variety
3.0	20	0.5	65	50
	20	1.0	70	45
	20	1.5	55	65
	20	2.0	45	35
	20	2.5	35	45
4.0	20	0.5	65	40
	20	1.0	70	45
	20	1.5	75	55
	20	2.0	85	65
	20	2.5	60	50
5.0	20	0.5	50	70
	20	1.0	55	75
	20	1.5	40	65
	20	2.0	50	80
	20	2.5	40	90

4.3.2 Number of shoots per explant

The effect of different concentrations of BAP and IBA on number of shoots per explant of Sagar variety of (Table 9) showed significant variations at 1% level. The highest number of shoots was produced by the treatment of 4.0 mg/L BAP+1.5 mg/L IBA (5.60 shoots per explant) (Plate 15). The treatment of 4.0 mg/L BAP + 0.5 mg/L IBA showed good number of shoots proliferations (3.80 shoots per explant). On the other hand, the lowest number of shoots (1.00 shoot per explant) was produced by the treatment combination of 3.0 mg/L BAP + 2.5 mg/L IBA.

In Sabri variety of Banana, the highest number of shoots per explant (Table 9) was produced by the treatment of 5.0 mg/L BAP+2.0 mg/L IBA (3.40 shoots per explant) (Plate 16). But the treatment of 5.0 mg/L BAP + 2.5 mg/L IBA showed good number of shoots proliferations (3.00 shoots per explant). On the other hand, the lowest number of shoots (1.60 shoots per explant) was produced by the treatment concentrations of 4.0 mg/L BAP + 2.0 mg/L IBA. The treatment 3.0 mg/L BAP + 2.5 mg/L IBA produced lower which was statistically identical with the treatment of 4.0 mg/L BAP +2.5 mg/L IBA. Iqbal *et al.* (2013) found that the highest number of

shoots was produced with 5.0 mg/L BAP +1.0 mg/L IAA + 10 % CW (10 shoots/explant) at 40 DAI and 6.0 mg/L BAP + 1.0 mg/L IAA +10% coconut water (8 shoots/explant) at 40 DAI. The result of current investigation is partially supported by Huq *et al.* (2012) where they found maximum number of shoots (10) per explant when cultured on MS + 4.0 mg/L BAP + 2.0 mg/L IAA + 13% (v/v) coconut water using Banana (*Musa* sp.) cv. Sabri. This variation might be due to the different concentrations of BAP and IBA and their interaction.

Table 9. Combined effect of BAP and IBA on the number of shoot in Sagar and Sabri varieties of Banana

BAP (mg/L)	IBA (mg/L)	Number of shoot	
		Sagar variety	Sabri variety
3.0	0.50	3.40 ±1.82bc	2.00 ±0.0b
	1.00	2.60 ±0.55bcde	1.80 ±0.45b
	1.50	2.20 ±0.45cdefg	1.80 ±0.45b
	2.00	2.00 ±0.0defg	2.00 ±0.0b
	2.50	1.00 ±0.0g	1.60 ±0.55b
4.0	0.50	3.80 ±0.84b	3.00 ±0.0a
	1.00	2.20 ±0.45cdefg	2.00 ±0.0b
	1.50	5.60±0.55a	2.00 ±0.0b
	2.00	2.80 ±0.84bcd	1.60 ±0.55b
	2.50	1.60 ±0.90defg	1.60 ±0.55b
5.0	0.50	2.00 ± 0.0defg	3.00 ±0.0a
	1.00	2.40 ±0.55cdef	2.00 ±0.0b
	1.50	2.00 ±0.0defg	2.00 ±0.0b
	2.00	1.40 ±0.55efg	3.40 ±0.55a
	2.50	1.20 ±0.45fg	3.00 ±0.0a
$LSD_{(0.01)}$		1.174	0.55
CV (%)		28.91	14.94
SE		0.312	0.146

Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT.

Plate 15. Multiple shoots produced in "Sagar" variety of Banana on MS supplemented with 4.0 mg/L BAP + 1.5 mg/L IBA at 4 WAI.

Plate 16. Multiple shoots produced in "Sabri" variety of Banana on MS supplemented with 5.0 mg/L BAP +2.0 mg/L IBA at 4 WAI.

4.3.3 Length of shoot (cm)

The significant variations were observed among different concentrations of BAP and IBA on growth parameter of Sagar variety of Banana such as the length of shoot (cm) in MS media (Table 10). The highest length of shoot was recorded in the combination of 4.0 mg/L BAP+2.5 mg/L IBA (5.55 cm) which was statistically similar with the treatment of 5.0 mg/L BAP +2.0 mg/L IBA (5.28 cm), 5.0 mg/L BAP+2.5 mg/L IBA (5.20 cm), 3.0 mg/L BAP + 2.5 mg/L IBA (5.27 cm) and 3.0 mg/L BAP + 0.5 mg/L IBA (5.02 cm). On the other hand, the lowest length of shoot (3.53 cm) was produced by the treatment of 4.0 mg/L BAP + 1.5 mg/L IBA which was statistically similar with the treatment of 4.0 mg/L BAP + 1.0 mg/L IBA (3.56 cm).

The highest length of shoot (5.15 cm) of Sabri variety of Banana (Table 10) was found in the treatment combination of 4.0 mg/L BAP+2.5 mg/L IBA which was statistically similar with the treatment combination of 4.0 mg/L BAP +2.0 mg/L IBA (4.46 cm), 5.0 mg/L BAP+ 1.5 mg/L IBA (4.50 cm), 5.0 mg/L BAP + 0.5 mg/L IBA (4.39 cm) and 3.0 mg/L BAP +1.5 mg/L IBA (4.35 cm). On the other hand, the lowest length of shoot (3.37 cm) was recorded in the treatment of 5.0 mg/L BAP+ 1.0 mg/L IBA. Al-amin *et al.* (2009) found the longest shoot (1.03, 2.45 and 3.38 cm) to the treatment combination of 7.5 mg/L BAP + 0.5 mg/L NAA at 10, 20 and 30 DAI, respectively. The findings of current investigation are not supported by Azam *et al.* (2010) where they found the addition of 0.1 mg/L IBA and 10% coconut water with 2.0 mg/L BAP to the medium increased shoot elongation and stimulated growth of the shoots, respectively using Banana (*Musa* sp.) cultivar BARI-1 (AAA genome, *sapientum* subgroup). George, *et al.* (2008) observed that low auxin in combination with high cytokinin induces auxilary shoot proliferation.

Table 10. Combined effect of BAP and IBA on length of shoot in Sagar and Sabri varieties of Banana

BAP (mg/L)	IBA (mg/L)	Length of shoot (cm)	
		Sagar variety	Sabri variety
3.0	0.50	5.02 ±0.33a	3.74 ±0.25bc
	1.00	3.95 ±0.18bcd	4.23 ±0.47bc
	1.50	4.25 ±0.25bc	4.35 ±0.51ab
	2.00	3.64 ±0.19cd	4.18 ±0.25bc
	2.50	5.27 ±0.37a	4.14 ±0.49bc
4.0	0.50	4.40 ±0.37b	4.10 ±0.33bc
	1.00	3.56 ±0.19d	3.75 ±0.27bc
	1.50	3.53 ±0.07d	3.65 ±0.34bc
	2.00	4.36 ±0.19b	4.46 ±1.0ab
	2.50	5.55 ±0.11a	5.15 ±1.02a
5.0	0.50	3.82 ±0.10bcd	4.39 ±0.28ab
	1.00	4.01 ±0.12bcd	3.37 ±0.34c
	1.50	3.72 ±0.32 bcd	4.50 ±0.0ab
	2.00	5.28 ±0.50a	3.92 ±0.40bc
	2.50	5.20 ±0.98a	3.71 ±16bc
$LSD_{(0.01)}$		0.607	0.821
CV (%)		8.26	11.87
SE		0.161	0.218

Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT.

4.3.4 Number of leaf

The significant variations were observed among different combinations of BAP and IBA on the number of leaf (Table 11). The highest number of leaf was produced by 3.0 mg/L BAP+0.5 mg/L IBA (3.30) which was statistically similar with the treatment of 4.0 mg/L BAP +1.5 mg/L IBA. On the other hand, the lowest number of leaf was produced by the treatment of 3.0 mg/L BAP+ 2.0 mg/L IBA (2.10).

Sabri varieties showed the highest number of leaf at 5.0 mg/L BAP+0.5 mg/L IBA (3.67) followed by (3.50) (Table 11). On the other hand, the lowest number of leaf was recorded 5.0 mg/L BAP+1.0 mg/L IBA (2.10) which was statistically identical with the treatment of 4.0 mg/L BAP+ 1.0 mg/L IBA. In the case of Sabri, the findings of the present study agree with the findings of Demissie, (2013) who found that the maximum number of leaves of plantain (*Musa* sp.) cv. Matoke at 10, 20, 30 and 60 DAI produced on the medium supplemented of with 5.0 mg/L BAP and 0.5 mg/L NAA are 1.67, 2.67, 3.67 and 4.33 per explant. Rahman *et al.* (2013) achieved that the average number of leaves produced in different concentrations of BAP, 2iP and Kin ranged from 0.44 to 2.69. The maximum number of leaves (2.69) was produced ($P<0.05$) under 2.0 mg/L 2iP while the lowest number was found under 1.0 mg/L Kin. Though the number of leaves increased with time in all treatments, the increasing rate was faster in BAP compared to 2iP and Kin.

Table 11. Combined effect of BAP and IBA on the number of leaf in Sagar and Sabri varieties of Banana

BAP (mg/L)	IBA (mg/L)	Number of leaf	
		Sagar variety	Sabri variety
3.0	0.50	3.30 ±0.30a	2.60 ±0.65bcd
	1.00	2.80 ±0.27abcd	2.60 ±0.42bcd
	1.50	2.40 ±0.41cd	2.80 ±0.67abcd
	2.00	2.10 ±0.22d	2.70 ±0.67abcd
	2.50	2.40 ±0.55bcd	2.60 ±0.65bcd
4.0	0.50	2.97 ±0.57abc	3.17 ±0.33abc
	1.00	3.13 ±0.30ab	2.10 ±0.22d
	1.50	3.23 ±0.33a	3.50 ±0.35ab
	2.00	2.20 ±0.45cd	3.20 ±0.76abc
	2.50	2.23 ±0.32cd	2.90 ± 0.22abcd
5.0	0.50	2.40 ±0.42bcd	3.67 ±0.77a
	1.00	2.40 ±0.55bcd	2.10 ±0.22d
	1.50	2.20 ±0.27cd	3.10 ±0.22abc
	2.00	2.40 ±0.41bcd	2.27 ±0.40cd
	2.50	2.30 ±0.45cd	2.91 ±0.30abcd
$LSD_{(0.01)}$		0.679	0.845
CV (%)		15.76	17.82
SE		0.181	0.252

Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT.

4.3.5 Length of leaf (cm)

Combined effect of BAP and IBA on the length of leaf (cm) has been found significant at 1% level in (Table 12). The highest length of leaves (3.74 cm) was exhibited by the treatment 3.0 mg/L BAP+ 0.5 mg/L IBA which was statistically similar with the treatment of 3.0 mg/L BAP+ 1.0 mg/L IBA (3.29 cm). On the other hand, shortest leaves (2.21 cm) were produced by the treatment of 3.0 mg/L BAP+ 1.5 mg/L IBA.

In Sabri variety of Banana the longest leaf was produced by the concentrations of 5.0 mg/L BAP+ 1.5 mg/L IBA (3.20 cm) (Table 12). The second longest leaf length was produced by the treatment of 5.0 mg/L BAP+0.5 mg/L IBA (2.95 cm). On the other hand, the lowest length of leaf was produced in 4.0 mg/L BAP+ 1.5 mg/L IBA (2.05 cm). The results of present experiment agree partially with the findings of Rahaman *et*

al. (2004) where they obtained longest leaf in the treatment 5.0 mg/L BAP (3.62 cm) followed by 1.5 mg/L NAA and 4.0 mg/L BAP (3.40 cm) using BARI Banana-1. But in Sagar variety, the results are not supported by the findings of Rahaman *et al.* (2004).

Table 12. Combined effect of BAP and IBA on length of leaf in Sagar and Sabri varieties of Banana

BAP (mg/L)	IBA (mg/L)	Length of leaf (cm)	
		Sagar variety	Sabri variety
3.0	0.50	3.74 ±0.12a	2.32 ±0.10cde
	1.00	3.29 ±0.25ab	2.25 ±0.15cde
	1.50	2.82 ±0.11bcd	2.34 ±0.24cde
	2.00	2.47 ±0.03cde	2.33 ±0.24cde
	2.50	2.50 ±0.45cde	2.72 ±0.21abcd
4.0	0.50	2.88 ±0.65bcd	2.19 ±0.30de
	1.00	2.99 ±0.37bc	2.53 ±0.08bcde
	1.50	2.77 ±0.25bcd	2.05 ±0.54e
	2.00	2.68 ±0.07cde	2.07 ± 0.07e
	2.50	2.57 ±0.31cde	2.74 ±0.13abc
5.0	0.50	2.35 ±0.25de	2.95 ±0.51ab
	1.00	2.47 ±0.18cde	2.89 ±0.25ab
	1.50	2.21 ±0.16e	3.20 ±0.41a
	2.00	2.97 ±0.29bc	2.89 ±0.25ab
	2.50	3.02 ±0.18 bc	2.21 ±0.17cde
$LSD_{(0.01)}$		0.48	0.52
CV (%)		10.29	10.02
SE		0.12	0.14

Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT.

4.3.6 Number of roots per explant

The effects of different concentrations of BAP and IBA on number of root of Sagar and Sabri varieties of Banana were statistically significant at 1% level of significance (Table 13). The highest number of root (5.00 and 5.20 per explant) was found in the treatment of 3.0 mg/L BAP+1.0 mg/L IBA in Sagar and Sabri varieties of Banana. On the other hand, the lowest numbers of roots (1.40 and 2.00 per explant) were produced by the treatment concentrations of 5.0 mg/L BAP+0.5 mg/L IBA in two Banana varieties. Vigorous roots with plenty of hairy roots of Sagar and Sabri variety of Banana were grown on MS media supplemented with 3.0 mg/L BAP+1.0 mg/L IBA (Plate 17 &18). Gubbuk and Pekmezc (2004) found that supplementation of 2.0 µM TDZ, and 1.0 µM IAA or 20.0 µM BAP and 1.0 µM IAA on MS medium, followed 5.0 g/L charcoal were the best combinations for the *in vitro* root regeneration of banana types.

Table 13. Combined effect of BAP and IBA on number of root in Sagar and Sabri variety of Banana

BAP (mg/L)	IBA (mg/L)	Vigor of regenerated Root	Number of root	
			Sagar variety	Sabri variety
3.0	0.5	+++	3.40 ±0.90a	3.40 ±0.90a
	1.0	+++	5.00 ±0.70a	5.20 ±0.84a
	1.5	++	2.80 ±0.84a	2.80 ±0.84a
	2.0	+	2.20 ±0.84a	2.20 ±1.1a
	2.5	++	2.40 ±0.55a	2.20 ±0.45a
4.0	0.5	++	2.60 ±0.55a	2.60 ±0.55a
	1.0	+	2.00 ±0.0a	2.20 ±1.1a
	1.5	++	2.40 ±0.55a	2.40 ±0.55a
	2.0	+++	3.20 ±0.55a	3.20 ±0.45a
	2.5	+	2.40 ±0.55a	2.40 ±0.55a
5.0	0.5	+	1.40 ±0.55a	2.00 ±0.0a
	1.0	+	2.20 ±0.45a	2.40 ±0.55a
	1.5	+	2.00 ±0.70a	2.20 ±0.84a
	2.0	++	2.80 ±0.84a	2.40 ±0.55a
	2.5	++	2.60 ±0.55a	2.40 ±0.55a
$LSD_{(0.01)}$			3.36	2.92
CV (%)			23.92	24.08
SE			0.89	0.78

Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT.

+ = Less vigorous growth, ++ = Good growth and vigor, +++ =Best growth and vigor

Plate 17. Vigorous roots of "Sagar" variety of Banana grown on MS supplemented with 3.0 mg/L BAP + 1.0 mg/L IBA.

Plate 18. Vigorous roots of "Sabri" variety of Banana grown on MS supplemented with 3.0 mg/L BAP + 1.0 mg/L IBA.

4.3.7 Length of root (cm)

The significant variations were observed at different concentrations of BAP and IBA on length of root (cm) of Sagar varieties (Table 14). The highest length of root (6.61 cm) was produced by the treatment of 3.0 mg/L BAP+1.0 mg/L IBA (Plate 19). On the other hand, the minimum length of root (2.79 cm) was produced by the concentrations of 5.0 mg/L BAP + 0.5 mg/L IBA. In Sabri varieties the highest length of root (6.72 cm) was produced by the treatment combination of 3.0 mg/L BAP+0.5 mg/L IBA (Table 14, Plate 20). On the other hand, the minimum length of root (3.06 cm) was produced by the treatment concentrations of 5.0 mg/L BAP + 0.5 mg/L IBA. The result of current investigation is not supported by Mala *et al.* (2005). They observed that high auxin in combination with low cytokinin induces root formation. This variation may be due to the interaction of both endogenous and exogenous hormones (BAP and IBA).

Table 14. Combined effect of BAP and IBA on length of root for Sagar variety of Banana

BAP (mg/L)	IBA (mg/L)	Length of root (cm)	
		Sagar variety	Sabri variety
3.0	0.5	5.65 ±0.15ab	5.93 ±0.17ab
	1.0	6.61 ±0.16a	6.72 ±0.50a
	1.5	5.21 ±0.15abc	5.62 ±0.28ab
	2.0	5.13 ±0.15abc	5.37 ±0.32ab
	2.5	4.32 ±0.15abc	4.54 ±0.38ab
4.0	0.5	4.50 ±0.15abc	5.12 ±0.47ab
	1.0	4.99 ±0.13abc	5.37 ±0.12ab
	1.5	4.38 ±0.13abc	4.82 ±0.54ab
	2.0	4.16 ±0.10abc	5.12 ±0.11ab
	2.5	3.39 ±0.09bc	4.21 ±0.13ab
5.0	0.5	2.79 ±0.09c	3.06 ±0.19b
	1.0	4.34 ±0.09abc	4.45 ±0.32ab
	1.5	4.24 ±0.09abc	4.28 ±0.08ab
	2.0	3.56 ±0.09bc	4.84 ±0.43ab
	2.5	3.58 ±0.03bc	4.37 ±0.15ab
$LSD_{(0.01)}$		2.37	2.59
CV (%)		8.31	6.48
SE		0.63	0.69

Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT.

Plate 19. Longest root of "Sagar" variety of Banana grown on MS supplemented with 3.0 mg/L BAP + 1.0 mg/L IBA.

Plate 20. Longest root of "Sabri" variety of Banana grown on MS supplemented with 3.0 mg/L BAP + 1.0 mg/L IBA.

4.5 *Ex vitro* acclimatization and establishment of plantlets on soil

After sufficient shoot and root development at 6 weeks of culture, the small plantlets were taken out from culture vessel carefully without damaging any roots. Excess media around the root was washed off by running tape water to prevent further microbial infection. The plantlets were then transplanted in plastic pots filled with sterilized soil: cowdung (1:1) and soil mixture were treated with a solution of 1% IBA due to proper rooting in plastic pots. Immediately after transplantation the plantlets were irrigated with a fine spray of water. Occasional spray of water was done to prevent sudden desiccations and maintain high humidity around the plantlets and the plantlets were placed in to the controlled environment for proper hardening (Plate 21). The highest survival rate 95.00% found in Sagar varieties and the survival rate of Sabri varieties was 90.00% (Table 15).

Table 15. Survival rate of *in vitro* regenerated plants of two Banana varieties

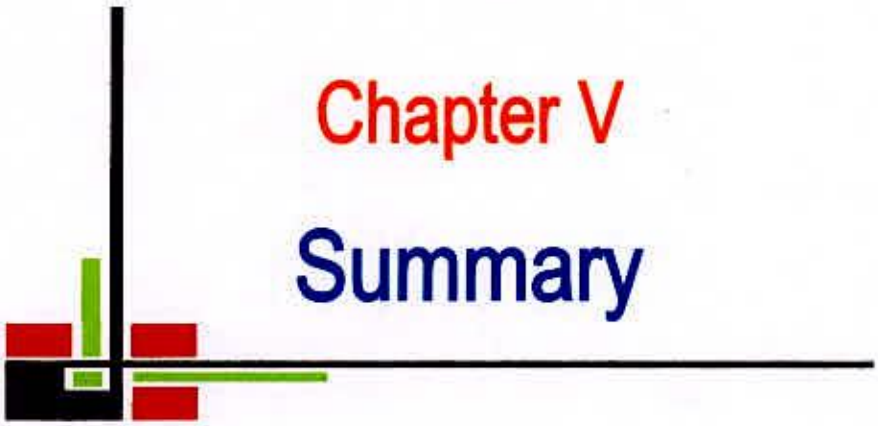
Acclimatization	Variety	No. of plants transplanted	No. of plants survived	Survival rate (%)
Initially plastic pots of plantlet in controlled environment	Sagar	20	19	95.00
	Sabri	20	18	90.00
Subsequently when moved to soil in open atmosphere	Sagar	19	18	94.74
	Sabri	18	16	88.89

After 20 days of hardening the plantlets were transplanted to soil (Plate 22). As soon as new leaves flushed, plants were watered with ordinary tap water. Gradually the plantlets were adapted to the soil. In the open atmosphere, the survival rate of Sagar varieties was 94.74% which was higher than the survival rate of 88.89% in Sabri varieties (Table 15). Mortality of the plantlets occurred during transplantation of plantlets due to shifting between containers, injuries to the root system and excessive evaporation. Rahman *et al.* (2013) found that the survival rate was around 90% in Banana (*Musa* spp.) cv. Agnishwar to the main field.

Plate 21. Hardening of the regenerated "Sagar" and "Sabri" varieties of Banana plantlets.

Sagar **Sabri**

Plate 22. Established "Sagar" and "Sabri" varieties of Banana in plastic pots in natural environment.



Chapter V

Summary

CHAPTER V

SUMMRRY

The experiment was conducted at the Biotechnology Laboratory, Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, during the period of October 2013 to August 2014 to investigate the effect of different concentrations of BAP and IBA singly and in combination, on regeneration, shoot proliferation and root formation in two varieties of Banana (Sagar and Sabri). Shoot tips were collected from Banana suckers of three months as explants.

Shoot tips were excised from mother plant and transferred to MS medium supplemented with hormone and also in control (0.0 mg/L). Regeneration of shoot tips resulted in globular ball like structure within 7 to 10 days of inoculation in medium containing different concentrations of BAP and combinations BAP and IBA. From this globular ball like structure adventitious plantlets were developing.

In experiment 1, the effects of different concentrations of BAP (0.0, 1.0, 2.0, 3.0 4.0 and 5.0 mg/L) were studied on shoot proliferation of Sagar and Sabri varieties of Banana. The results of this experiment revealed that the 4.0 mg/L BAP performed as the best treatment for Sagar variety of Banana and 5.0 mg/L BAP for Sabri variety of Banana on multiple shoot proliferation.

In the case of percent response of explants, the highest percent response of explant (88 and 84%, respectively) was observed with 4.0 mg/L BAP and 5.0 mg/L in Sagar and Sabri varieties of Banana and the lowest (40% and 44%) was observed with control (0.0 mg/L) in both varieties. In both Sagar and Sabri, the maximum 22.0 and 28.0 days, respectively were recorded for shoot induction in control and the minimum 7.80 days were required at 4.0 mg/L BAP in Sagar. For Sabri, the minimum 9.40 days were required for the same at 5.0 mg/L BAP.

The treatment of 4.0 mg/L BAP for Sagar showed highest number of shoots per explant (2.60, 4.60 and 6.80) and in Sabri 5.0 mg/L BAP showed same (2.00, 3.00

and 3.40) at 2, 4 and 6 WAI, respectively. In both the cases, lowest number of shoots was observed in control (1.00, 1.00 and 1.00 shoots per explant). In Sagar, the highest length of shoots as 3.69, 5.01 and 8.31 cm and in Sabri 3.22, 4.09 and 6.19 cm were observed in 4.0 mg/L at 2, 4 and 6 WAI. For Sagar, the lowest length of shoot (1.18, 1.50 and 2.27 cm) and that of Sabri 1.66, 1.77 and 2.30 cm were observed in control (0.0 mg/L) at 2, 4 and 6 WAI, respectively, which was statistically different from all other treatments. Considering the per cent increase of shoot length, the highest increase 212.71, 234.00 and 266.08% in 4.0 mg/L and that of the lowest 143.22, 150 and 143.17% in 1.0 mg/L were observed at 2, 4 and 6 WAI, respectively for Sagar. For Sabri, the highest increase (93.98, 131.07 and 169.13%, respectively) was observed in 4.0 mg/L whereas the minimum was observed in 2.0 mg/L (56.63, 87.01 and 132.61%) at 2, 4 and 6 WAI.

BAP at 4.0 mg/L showed highest number of leaf as 3.01, 3.29 and 4.24 in Sagar variety and with 5.0 mg/L as 2.54, 3.38 and 4.49 in Sabri variety of Banana at 2, 4 and 6 WAI, respectively. The lowest number of leaf (0.60, 1.00 and 1.60) of Sagar and that of Sabri (0.60, 1.00 and 1.80) was observed in control (0.0 mg/L) at 2, 4 and 6 WAI, respectively. Considering the percent increase of number of leaf over control, 4.0 mg/L treatment showed the highest increase (401.67, 229.00 and 165.00% respectively) and minimum increase over control was observed in 1.0 mg/L (100.00, 70.00 and 83.13%, respectively) at 2, 4 and 6 WAI, respectively of Sagar variety of Banana. Sabri variety of Banana showed the maximum increase (323.33, 238.00 and 149.44%) in 5.0 mg/L BAP and the lowest (133.33 and 75.00%, respectively) in 1.0 mg/L at 2 and 6 WAI. The highest length of leaf (2.01, 2.77 and 4.25 cm) of Sagar variety of Banana was observed in 3.0 mg/L BAP and that of Sabri variety (2.35, 2.92 and 4.44 cm) was observed in 5.0 mg/L at 2, 4 and 6 WAI, respectively. The highest increase of leaf by length over control (34.00, 174.26 and 150.00 %, respectively) was observed in 3.0 mg/L BAP of Sagar variety and for Sabri variety (335.19, 194.95 and 149.44%, respectively) was in 5.0 mg/L BAP at 2, 4 and 6 WAI, respectively.

In experiment 2, effects of different concentrations of IBA on root formation of the micropropagated shoots in Sagar and Sabri varieties of Banana were studied. In Sagar and Sabri, the highest numbers of days (15.20 and 14.20) were required in control and the lowest numbers of days (7.60 and 8.00) were needed in 1.5 mg/L IBA

for root formation. The highest number of roots per explants (2.00, 3.20 and 5.20 per explant) was produced by 0.5 mg/L of IBA at 2, 4 and 6 WAI, respectively and the lowest number of root was produced by the control in Sagar. In the case of Sabri, the highest number of roots per explant as 2.20, 3.40 and 3.40 was observed in 1.5 mg/L of IBA at 2, 4 and 6 WAI, respectively and the lowest number of roots was recorded in control. The highest length of roots (2.23, 3.15 and 4.29 cm) of Sagar and that of Sabri (1.74, 2.40 and 3.65 cm) was observed in 0.5 mg/L IBA at 2, 4 and 6 WAI, respectively.

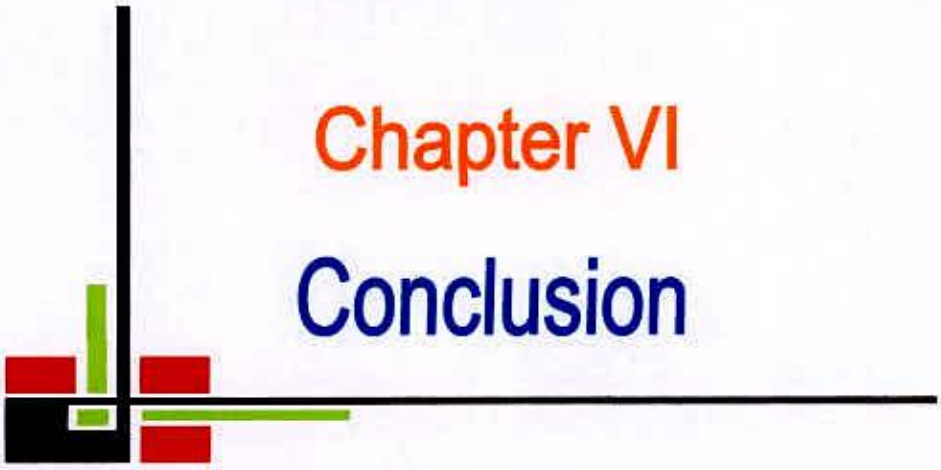
In experiment 3, combined effects of BAP and IBA on shoot and root regeneration in Sagar and Sabri varieties of Banana were studied. In combination of BAP and IBA, the highest percent response of explants (85% and 90%) was observed in Sagar and Sabri varieties with 4.0 mg/L BAP+2.0 mg/L IBA and 5.0 mg/L BAP+2.5 mg/L IBA. In both varieties, the lowest response percent (35%) was observed in concentration of 3.0 mg/L BAP+2.5 mg/L IBA and 3.0 mg/L BAP +2.0 mg/L IBA.

The highest number of shoots per explant (5.60) in Sagar was produced at 4.0 mg/L BAP+1.5 mg/L IBA and the lowest number of shoots per explant (1.0) was produced in 3.0 mg/L BAP+2.5 mg/L IBA. In Sabri variety the highest number of shoots (3.40 shoots per explant) was produced by 5.0 mg/L BAP+2.0 mg/L IBA and the lowest number of shoot (1.60 shoots per explant) was produced by the treatment concentrations of 4.0 mg/L BAP+2.0 mg/L IBA which was statistically similar with 4.0 mg/L BAP+2.5 mg/L IBA and 3.0 mg/L BAP+2.5 mg/L IBA. The highest length of shoot (5.55 cm) of Sagar was produced at 4.0 mg/L BAP+2.5 mg/L IBA and the lowest length (3.53 cm) of shoot was produced by 4.0 mg/L BAP+1.5 mg/L IBA. The highest length of shoot (5.15 cm) of Sabri variety of Banana was produced by the treatment concentrations of 4.0 mg/L BAP+2.5 mg/L IBA and the lowest length (3.37 cm) of shoot was produced by the treatment of 5.0 mg/L BAP+1.0 mg/L IBA. The highest number of leaf (3.30) of Sagar was produced by 3.0 mg/L BAP+0.5 mg/L IBA and the lowest number of leaf (2.10) was produced by 3.0 mg/L BAP+2.0 mg/L IBA. Sabri varieties of Banana showed the highest number of leaf (3.67) at 5.0 mg/L BAP+0.5 mg/L IBA and that of lowest (2.10) was produced at 5.0 mg/L BAP+1.0 mg/L IBA. In Sagar the highest length of leaf (3.74 cm) was observed at 3.0 mg/L BAP+0.5 mg/L IBA and the lowest length of leaf (2.21 cm) was produced by the treatment of 5.0 mg/L BAP+ 1.5 mg/L IBA. In Sabri variety of Banana the highest

length of leaf (3.20 cm) was produced by 5.0 mg/L BAP+ 1.5 mg/L IBA and the lowest length of leaf (2.05 cm) was produced by the treatment of 4.0 mg/L BAP+ 1.5 mg/L IBA.

In combination of IBA and BAP, the highest number of roots (5.00 and 5.20 per explant) and root length (6.61 and 6.72 cm) were produced by the treatment of 3.0 mg/L BAP+1.0 mg/L IBA in both Sagar and Sabri varieties of Banana. On the other hand, the minimum number of roots (1.40 and 2.00 per explant) and root length (2.79 and 3.06 cm) were at 5.0 mg/L BAP + 0.5 mg/L IBA in two Banana varieties.

For acclimatization, plantlets were transplanted from culture media to soil in plastic pots in controlled environment, where the higher survival rate (95.00%) was found in Sagar varieties and the lower survival rate (90.00%) was found in Sabri varieties. After hardening, plantlets were transferred to open atmosphere, where the survival rate of Sagar varieties was 94.74% which was higher than the survival rate of 88.89% in Sabri varieties.



Chapter VI

Conclusion

CHAPTER VI
CONCLUSION

Following conclusions can be made from the present study:

i. A micropropagation protocol has been developed in Sagar and Sabri varieties of Banana.

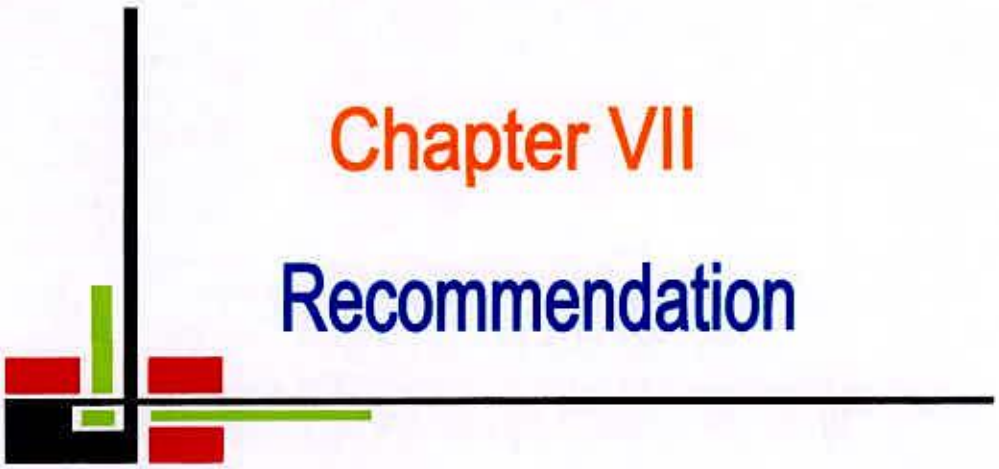
ii. Overall moderate higher dose (4.0 mg/L BAP) showed better response *in vitro* regeneration in Banana.

iii. Combined effect of BAP and IBA seems to be better than individual response of either BAP or IBA based on average performance of growth parameters.

iv. This protocol has the applicability *in vitro* rapid propagation of Banana.

Chapter VII

Recommendation



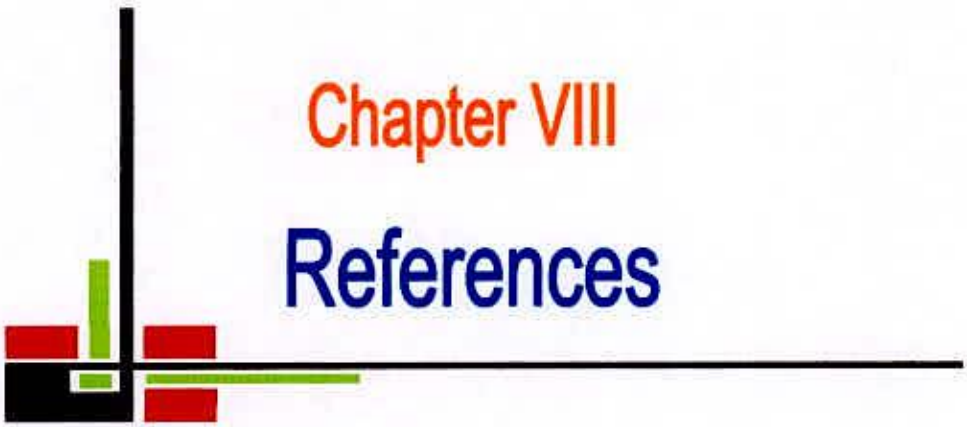
CHAPTER VII
RECOMMANDATION

Based on above conclusion following recommendation can be made.

i. Very few types and levels of hormone have been used which needs to be extended for *in vitro* regeneration in Sagar and Sabri to identify further better combination if any.

ii. The research was done with only two Banana varieties, Sagar and Sabri, which also necessitates to be widened in other demanding varieties for ultimate improvement of Banana in Bangladesh.

iii. The research being conducted with limited time duration, further extension and continuation of the research to assess field performance should be carried out.



Chapter VIII

References

CHAPTER VIII
REFERENCES

Abeyaratne, W. M. and Lathiff, M. A. 2002. *In vitro* propagation of 'Rathambala' (*Musa* AAA) and the occurrence of phenotypic variations in the pseudostem. Annals of the Sri Lanka Department of Agriculture (LKA). **4**: 191-197.

Agrios, G. N. 2005. Plant Pathology. Fifth Edition, Elsevier Academic Pess, California. p.922

Ahirwar, M. K., Mondal, S., Singh, M. K., Sen, C. and Singh, R. P. 2012. A high frequency plantlets regeneration protocol for Banana (*Musa paradisiaca* L.) micropropagation. *The Asian J. Hort.*7 (2): 397-401.

Al-amin, M., Karim, M. R., Amin, M. R., Rahman, S. and Mamun, A. N. M. 2009. *In vitro* Micropropagation of Banana (*Musa* sp.). *Bangladesh J. Agril. Res.* **34**(4): 645-659.

Ali, A., Sajid, A., Naveed, N.H., Majid, A., Saleem, A., Khan, U. A., Jafery, F.I., and Naz, S. 2011. Initiation,proliferation and development of micrpropagation system for mass scale production of banana through meristem culture. *African J. Biotech.* **10** (70):15731-15738.

Azam, F. M. S., Islam, S., Rahmatullah, M. and Zaman, A. 2010. Clonal propagation of Banana (*Musa* sp.) cultivar 'BARI-1' (AAA genome, sapientum subgroup). *Acta Hort.* p.879

Bairu, M.W., Stirk, W. A., Dolezal, K. and Staden, J. V. 2008. The role of topolins in micropropagation and somaclonal variation of Banana cultivars 'Williams' and 'Grand Naine' (*Musa* sp. AAA). *Plant Cell Tiss. Org. Cult.* **95**: 373-379. DOI 10.1007/s11240-008-9451-4

BBS. 2012. Statistics Year Book of Bangladesh, Bangladesh Bureau of Statistics, Ministry of Planning, Government of the people's Republic of Bangladesh, Dhaka.

Berg, L. A. and Bustamante, M. 1974. Heat treatment and meristem culture for the production of virus free Bananas. *Phytopathol.* **64**: 320-322.

Bhosale, U. P., Dubhashi, S. V., Mali, N. S. and Rathod, H. P. 2011. *In vitro* shoot multiplication in different species of Banana. *Asian J. Plant Sci. Res.* 2011. **1** (3):23-27.

Buah, J. N., Danso, E., Taah, K. J., Abole, E. A., Bediako, E. A., Asiedu, J., Baidoo, R. 2010. The Effects of Different Concentrations Cytokinins on the *in vitro* Multiplication of Plantain (*Musa* sp.). *Biotech.* **9**(3): 343-347.

Chariya, L. D. 2012. Physiological, Biochemical and Molecular Marker Studies in *Musa* sp., PhD thesis. Department of Biosciences, Saurashtra University, Rajkot, India.

CGIAR. 2011. Research and Impact: Area of Research-Banana. Acessed 22/09/2011. www. cigar.org/impact/research/Banana.html.

Coasta, F. H. D. S., Pasqual, M., Pereira, J. E. S. and Mauro de Castro, E. 2009. Anatomical and Physiological Modifications of Micropropagated 'Caipira' Banana Plants under Natural Light. *Sci. Agric.* (Piracicaba, Braz.). **66** (3): 323-330.

Cronauer, S. S. and A. D. Krikorian.1984a. Multiplication of Musa from excised stem tips. *Ann. Bot.* **53**(3): 32 1-328.

Cronauer, S. S. and Krikorian, A. D. 1984b. Aseptic culture techniques for Banana and plantain improvement. *Econ. Bot.* **38**: 322-33. DOI10.1007/BF02859010

Darvari, F. M., Sariah, M., Puad, M. P. and Maziah, M. 2010. Micropropagation of some Malaysian Banana and plantain (*Musa* sp.) cultivars using male flowers. *African J. Biotech.* **9**(16): 2360-2366.

Demissie, A. G. 2013. Effects of different combinations of BAP (6-benzyl amino purine) and NAA (naphthalene acetic acid) on multiple shoot proliferation of plantain (*Musa* spp.) cv. Matoke from meristem derived explant. *Academia J. Biotech.* **1**(5): 071-080. DOI: http://dx.doi.org/10.15413/ajb.2012.0110.

Dhed'a, D., Dumortier, F., Panis, B., Vuylsteke, D. and De Langhe, E. 1991. Plant regeneration in cell suspension cultures of cooking Banana 'Bluggoe'cultivar (*Musa* sp. ABB group). *Fruits.* **46**: 125-135.

Elisama, M. L. E., Raymundo, E. V., Arturo, V. V. V.,Virginia, C. A. G. and Luis, C. S. J. 2013. Acclimatization of micropropagated *Musa cavendischii* cultivar roatan plants submitted to doses of fertigation and auxin. *African J. Agrl.* **8**(43):5335-5340. DOI:10.5897/AJAR12.788 http://www.academicjournal. org/AJAR.

Emaga, T. H., Andrianaivo, R. H. Wathelet, B. J. T. and Paquot, M. 2008. Effects of the stage of maturation and varieties on the chemical composition of the Banana and plantain peels. *Food Chem.* **103**: 590-600.

Faisal, S. M., M. A. Hoque and A. Quasem. 1998. Field performance of *in vitro* plantlets against normal suckers of Banana (*Musa sapientum*) cv. Champa. *Plant Tiss. Cult.* **8**(2): 125-129.

FAO.2010. Statistics web site http://www.fao.org, accessed on 04/10/2012.

FAOSTAT. 2011. ProdSTAT: Crops, Food and Agriculture Organization. Available: www.faostat.org, retrieved on 24 February 2012.

Farahani, F., Aminpoor, H., Sheidai, M., Noormohammadi, Z., Mazinani, M. H. 2008. An improved system for *in vitro* propagation of Banana (*Musa acuminate* L.) Cultivars. *Asian J. Plant Sci.* **7**(1): 116-118.

Fernandez, M. B. M., Vargas, L. E. C., Montero, D. C., Garcia, A. C. Lopez, D. M. and Alvarez, S. P. 2014. Potentiality of Vermicompost Humic Acids in Banana *in vitro* Micropropagation Clone: Enano Guantanamero. *J. Environ. Sci. and Eng.* A **2**: 677-685.

George, E. F., Hall, M. A. and Klerk, G. J. 2008. Plant Propagation by Tissue Culture 3rd Edition *Spinger*. **1**:501.

Gilmar, R.Z., Gilberto, B.K., Jane, E.K and Elaine, C. 2000. Hormonal and histological studies related to *in vitro* banana bud formation. *Plant Cell Tiss. Org. Cult.* **63**: 187-192.

Gitonga, N. M., Ombori, O., Murithi, K. S. D. and Ngugi, M. 2010. Low technology tissue culture materials for initiation and multiplication of Banana plants. *African Crop Sci. J.* **18** (3): 243 – 251.

Goswami, N. K. and P. J. Handique, P. J. 2013. Explants Size Response to *in vitro* Propagation of Musa (Aaa Group) 'Amritsagar' Musa (Aab Group) 'Malbhog' and Musa (Aab Group) 'Chenichampa' Banana. *Indian J. Appl. Res.* **3**(8), pp.40-43

Gubbuk, H. and Pekmezc, M. 2004. *In vitro* Propagation of Some New Banana Types (*Musa* spp.) *Turk J. Agric*. **28**: 355-361

Guerrero (online). 2012. Diary of Invention (Agenda Innovacion), http://fundacion produce gro.org.mx/content/uploads/2012/05/12Pl%C3%A1tano.pdf

Habiba, U., Reza, S., Saha, M. L., Khan, M. R. and Hadiuzzaman, S. 2002. Endogenous bacterial contamination during *in vitro* culture of table Banana identification and prevention. *Plant Tiss. Cult.* **12** (2):117-124.

Hager, A., Debus, G., Edil H.G., Strosky, H., Serrano, R. 1991. Auxin induces exocytosis and the rapid synthesis of a high turnover pool of plasma membrane H+ ATPase. *Planta.* **185**: 527-537.

Heslop-Harrison and Schwarzacher, T. 2007. Domesticated, Genomics and Future for the Banana. Oxford journals: *Ann. of Bot.* **100**: 1073-1084.

Huq, A., Akter, S., Islam, S. and Khan, S. 2012. *In vitro* plant regeneration in Banana (*Musa* spp.) cv. Sabri. *Bangladesh J. Sci. Ind. Res.* **47**(2): 143-146.

Iqbal, M. M., Muhammad, A., Hussain, I. and Bilal, H. 2013. Optimization of *In Vitro* Micropropagation Protocol for Banana (*Musa Sapientum* L.) Under Different Hormonal Concentrations and Growth Media. *Int. J. Agril. Innov. Res.* **2**(1): 2319-1473.

Jarret, R. L., Rodriguez, W. and Fernandez, R. 1985. Evaluation, tissue culture propagation, Dissemination of 'Saba' and 'Pelipita' plantain in Costa-Rica. *Sci. Hortic.* **25**:137-147.

Kalimuthu, K., Saravanakumar, M. and Senthilkuma, R. 2007. *In vitro* micropropagation of *Musa sapientum* L. (Cavendish Dwarf). *African J. Biotech.* **6**(9): 1106-1109.

Karim, M. R., Malek, M. A., Rahman, S., Al-amin, M. and Amin, M., R. 2009. *In vitro* propagation of Banana. *Bangladesh J. Agril. Res.* **34**(2): 269-278.

Kulkarni, V. M., Suprasanna, P., Ganapathi, T. R, Bapat, V. A., Rao, P. S. 2004. Differential effect of igenome and cytokinin on shoot-tip cultures of Indian Banana cultivars. *Physiol. Mol. Biol. Plant.* **10**: 75-81.

Kulkarni, V. M., Suprasanna, P., Bapat, V. A. 2006. Plant regeneration through multiple shoot formation and somatic embryogenesis in a commercially important and endangered Indian Banana cv. Rajeli. *Curr. Sci.* **90**: 842-846.

Kumar, K. P. S., Bhowmik, D., Duraivel, S. and Umadevi, M. 2012. Traditional and medicinal uses of Banana. *J. Pharma. and Phytochem.* **1**: 51-63.

Lee, S. 2005.Thidiazuron in the Improvement of Banana Micropropagation Taiwan Banana Research Institute, Chiuju, Pingtung,Taiwan. pp.67-75

Mala, J., Gaudinova, A., Dobrev, P., Eder, J. and Cvikrova, M. 2005. Role of phytohormones in organogenic ability of elm multiplicated shoots. *Biol Planta*. **50**(1): 8-14.

Ma, S. S. and Shii, C. T. 1974. Growing Banana plantlets from adventitious buds. *J. Chin. Soc. Hort. Sci*. **20**: 6-12.

Ma, S. S. and Shii, C. T. 1972. *In vitro* formation of adventitious buds in Banana shoot apex following decapitation. *J. Chin. Soc. Hort. Sci*. **18**:135-142

Madhulatha, P., Anbalagan, M., Jayachandran, S. and Sakthivel, N. 2004. Influence of liquid pulse treatment with growth regulators on *in vitro* propagation of Banana. *Plant Cell Tiss. Org. Cult*. **76**:189-191.

Madhulatha, P., Kirubakaran, S. I. and Sakthivel, N. 2006. Effect of carbon sources and auxin on *in vitro* propagation of Banana. *Biol. Planta*. **50**(4):782-784.

Martin, K. P., Pachathundikandi, S. K., Zhang, C. L., Slater, A., Joseph, M. 2006. RAPD Analysis of a variant of Banana (*Musa* spp.) cv. Grande Naine and its propagation via shoot tip culture. *In vitro Cell. Dev. Biol. Plant*. **42**:188–192.

Morais-Lino, L. S., Serejo, J. A. S., Silva, S. O., Santana, J. R. F. and Kobayashi, A. K. 2008. Cell suspension culture and plant regeneration of a Brazilian plantain, cultivar Terra. *Pesq. Agropec. Bras*. Brasília. **43** (10):1325-1330

Morfeine, E. A. 2014. Effect of Sucrose and Glucose Concentrations on Micro-propagation of *Musa* spp. cv.Grand Naine. *J. Appl. and Ind. Sci*. **2** (2): 58-62.

Molla, M. M. H., Khanam, M. D., Khatun, M. M., Al-Amin, M. and Malek, M. A. 2004. *In vitro* rooting and ex vitro plantlet establishment of BARI Banana (*Musa spp.*) as influenced by different concentration of IBA (Indole 3-butyric Acid) *Asian J. Plant Sci.* **3** (2):196-199.

Muhammad, A., Hussain, I., Naqvi, S. M. S. and Rashid, H. 2004. Banana Plantlet Production through Tissue Culture. *Pak. J. Bot.* **36**(3): 617-620.

Muhammad, A., Hussain, I., Rashid and Quraishi, A. 2000. Enhanced *in vitro* multiplication of Banana (*Musa* sp.) *Plant Tiss. Cult.* **10** (2):11-117.

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

Musambiyama, T., Seshu, K.V. and Ngode, L. 2000. Evaluation of Banana cultivars for resistance to the Banana weevil and nematode control in western Kenya. *Acta Hort.* **540**:233–238.

Nelson, S. C., Ploetz, R. C. and Kepler, A. K. 2006. Species profiles for Pacific island agroforestry: Musa species (Banana and plantain). Musaceae (Banana family). http://www.agroforestry.net/tti/Musa-Banana-plantain.pdf.

Novak, F. J. 1992. *Musa* (Bananas and Plantains). In: Hammerschlag, F. A. and Litz, R. E. (eds), Biotechnology of Perennial Fruit Crops. CAB International, University Press, Cambridge. U. K. pp. 449-488.

Oliveira, R. P. D., Silveira, D. G., Oliveira, S. D. and Oliveira, E. S. D. 2001. BAP Concentration and tetraploid Banana micropropagation efficiency (AAAB group). *Sci. Agric.* Brazil. **58** (1):73-78.

Pua, E. C. 2007. Banana. In: Biotechnology in Agriculture and Forestry, Pua, E. C. and M. R. Davey (Eds.). *Springer*. **60**: 3-34.

Rahman, H., Hossain, M. and Islam, R. 2012. Micropropagation of Elite Genotypes of Banana: *In vitro* Mass Production of Banana (*Musa* spp.) Plants through Micropropagation and Somatic Embryogenesis. LAP LAMBERT Academic Publishing.

Rahman, M. Z., Nasiruddin, K. M., Amin, M. A., Islam, M. N. 2004. *In vitro* Response and Shoot Multiplication of Banana with BAP and NAA. *Asian J. Plant Sci.* **3**:406-409.

Rahman, M. Z., Sharoar, M.G., Matin, M. N., Rahman, M. H., Rahman, M. M.and Islam, M. R. 2006. High Frequency Plant Regenratioon of a Dessert Banana cv. Mehersagar for Commercial Exploitation. *Biotech.* **5**(3): 296-300.

Rahman, S., Biswas, N., Hassan, M. M., Ahmed, M. G., Mamun, A. N. K., Islam, M. R., Moni ruzzaman, M., and Haque, M. E. 2013. Micro propagation of Banana (*Musa* spp.) cv. Agnishwar by *In vitro* shoot tip culture. *Int. Res. J. Biotech.* **4**(4): 83-88.

Ray, T., Dutta, I., Saha, P., Das, S. and Roy, S. C. 2006. Genetic stability of three economically important micropropagated Banana (*Musa* sp.) cultivars of lower Indo-Gangetic plains, as assessed by RAPD and ISSR markers. *Plant Cell Tiss. Org. Cult.* **85**: 11-21.

Resmi, L. and Nair, A. S. 2007. Plantlet production from the male inflorescence tips of *Musa acuminata* cultivars from South India. *Plant Cell Tiss. Org. Cult.* **88**: 333-338.

Robinson, J. C. and De Villiers, E.A. 2007. The cultivation of Banana. Nelpcruit, South Africa: ARC-Institute for Tropical and Subtropical Crops. Robinson, J. C. 1996. Bananas and plantains, walling ford, UK: CAB International.

Rohr, R., Iliev, I., Scaltsoyiannes, A. and Tsoulpha, P. 2003. Acclimatization of micropropagated forest trees. *Acta Hort.* **616**: 59-69.

Roux, N., Baurens, F. C., Dolezel, J., Hribova, E., Heslop-Harrison, P., Town, C., Sasaki, T., Matsumoto, T., Aert, R., Remy, S., Souza, M. and Lagoda, P. 2008. Genomics of Banana and plantain (*Musa sp.*), major staple crops in the tropics. In: Moore P. H., Ming, R. (eds) Genomics of tropical crop plants. *Springer.* 83-111.

Roux, N., J. Dolezel, R. Swennen and F.J. Zapata-Arias. 2001. Effectiveness of three micropropagation techniques to dissociate cytochimeras in *Musa* sp. *Plant Cell Tiss. Org.Cult.* **66**: 189-197.

Sadik, K., Rubaihayo, P. R., Magambo, M. J. S. and Pillay, M. 2007. Generation of cell suspensions of East African highland Bananas through scalps. *African. J. Biotech.* **6**:1352-1357.

Santos, C. C. C. and Rodrigues, P. H. V. 2004. Somaclonal Variation Event on Micropropagated Pacovan Banana Seedling (*Musa* spp. Aab Group). Bragantia, Campina. **63** (2): p.201-205

Sasson, A. 1997. Importance of tropical and subtropical horticulture, future prospects of biotechnology in tropical and subtropical horticulture species. *Acta Hortic.* **460**: 12-26.

Scaranari, C., Leal, P. A. M. and Mazzafera, P. 2009. Shading and Periods of Acclimatization of Micropropagated Banana Plantlets cv.Grande Naine. *Sci. Agric.* (Piracicaba, Braz.). **66**(3):331-337.

Sholi, N. J. Y., Chaurasia, A., Agrawal, A. and Sarin, N. B. 2009. ABA enhances plant regeneration of somatic embryos derived from cell suspension cultures of plantain cv. Spambia (*Musa* spp.). *Plant Cell Tiss. Org. Cult.* **99**: 133-140. DOI: 10.1007/s11240-009- 9585-z.

Silva, D. S.; Bosisio A.; Boscarol B.; Beltzer, A.; Amsler, G. P. 1999. Aclimatação de mudas de bananeira (*Musa* sp.)"Prata"(AAB) em diferentes substratos. Revista Ceres. **46**:543-554, 1999.

Singh, H. P., Uma, S., Selvarajan, R. and Karihaloo, J. L. 2011. Micropropagation for Production of Quality Banana Planting Material in Asia-Pacific. Asia-Pacific Consortium on Agricultural Biotechnology (APCoAB), New Delhi, India.

Singh, M., Jaiswal, U. and Jaiswal, V. S. 2004. *In vitro* Regeneration and Improvement in Tropical Fruit Trees: An Assessment. In: Plant Biotechnology and Molecular Markers, Srivastava, P.S., A. Narula and S. Srivastava (Eds.). Anamaya Publishers, New Delhi, India. pp. 229-243.

Souza, A. S., Dantas, J. L. L., Souza, F.V.D., Cordeiro, Z. J. M., Silva Neto, S. P. 1997. Propagaçao. In: ALVES, E.J. (Org.) A cultura da Banana: aspectos técnicos, socioeconômicoe agroindustriais. Cruz das Almas: EMBRAPA-CNPMF. p.151-195.

Strosse, H., Andre, E., Sagi, L., Swennen, R. and Panis, B. (2008). Adventitious shoot formation is not inherent to micropropogation of banana as it is in maize. *Plant cell Tiss. Org. Cult.* **95**:321-332.

Strosse, H., I. Van, I. D. H. and Panis, B. 2004. Banana Cell and Tissue Culture-Review. In: Banana Improvement: Cellular, Molecular Biology and Induced Mutations, Jain, S.M. and R. Swennen (Eds.), Science Publishers, Inc. pp:1-12.

Swamy, R. D., Rao, N. K. S. and Chacko, E.K.1983.Tissue-culture propagation of Banana. *Sci. Hortic.***18**: 247-252.

Villalobos, M. R. and Garcia, E. D. 2008. Obtainment of embryogenic cell suspensions from scalps of the Banana Cien-Bta-03 (*Musa* spp., AAAA) and regeneration of the plants. *Elect. J. Biotechnol.* **11**: 1-10. DOI: 10.2225/vol11-issue5-fulltext-3.

Vishwas, B. C. P., Chetan, D. A. and Gaikwad, D. K. 2010. Field performance of *in vitro* propagated Banana plants from 8th and 15th subculture. *Int. J. Adv. Biotech. Res.* **1**(2): 96-103

Vora, N. C. and Jasrai, Y. T. 2012. Natural And Low-Cost Substitutes of Synthetic PGR For Micropropagation of Banana. *Cibtech J. Biotech.* **2**(1): 9-13

Vuylsteke, D. and De Langhe, E. 1985. Feasibility of *in vitro* propagation of Banana and plantains, Tropical Agriculture (Trinidad). **62**: 323-328.

Vuylsteke, D. R. and R. Ortiz, R. 1996. Field performance of conventional vs. *in vitro* propagules of plantain (*Musa* sp., AAB group). *Hortic. Sci.* **31**: 862-865.

Wanja, I. 2010. Management practices and opportunities in East Africa highland Bananas (*Musa* sp. AAA-EA). PhD thesis. Wageningen University.

Appendices

APPENDICES

Appendix I. Effect of BAP on the percent response of explant in two varieties of Banana

BAP (mg/L)	*Percent response of explant (%)	
	Sagar variety	Sabri variety
0.0	40	44
1.0	48	52
2.0	52	56
3.0	68	64
4.0	88	72
5.0	76	84

*Twenty five explants were inoculated in each treatment.

Appendix II. Effect of BAP on number of shoot in Sagar variety of Banana

BAP (mg/L)	No. of shoot at 2 WAI*	% Increase over control	No. of shoot at 4 WAI*	% Increase over control	No. of shoot at 6 WAI*	% Increase over control
0.0	1.00 ±0.0 b	-	1.00 ±0.0d	-	1.00 ±0.0e	-
1.0	1.00 ±0.0b	0	1.60±0.55cd	60.00	2.20±0.45d	120.00
2.0	1.00±0.0b	0	2.00±0.0bcd	100.00	2.40±0.55d	140.00
3.0	2.20±0.45a	120	3.00 ±0.71b	200.00	5.00±0.71b	400.00
4.0	2.60±0.55a	160	4.60 ±0.89a	360.00	6.80±1.10a	580.00
5.0	1.40±0.55b	40	2.60±0.55bc	160.00	3.60±0.55c	260.00
$LSD_{(0.01)}$	0.65		0.996		1.142	
CV (%)	23.81		22.81		18.44	
SE	0.16		0.25		0.29	

*WAI = Weeks after inoculation. Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT.

Appendix III. Effect of BAP on number of shoot in Sabri variety of Banana

BAP (mg/L)	No. of shoot at 2 WAI*	% Increase over control	No. of shoot at 4 WAI*	% Increase over control	No. of shoot at 6 WAI*	% Increase over control
0.0	1.00 ±0.0b	-	1.00 ±0.0c	-	1.00 ±0.0c	-
1.0	1.00 ±0.0b	0	1.40±0.55c	40.00	2.00 ±0.0b	100.00
2.0	1.00 ±0.0b	0	1.20±0.45c	20.00	2.00 ±0.0b	100.00
3.0	1.60±0.55ab	60	2.00 ±0.0b	100.00	2.4 ±0.55b	140.00
4.0	1.40±0.55b	40	2.00 ±0.0b	100.00	3.00 ± 0.0a	200.00
5.0	2.00 ±0.0a	100	3.00 ±0.0a	200.00	3.40±0.55a	240.00
$LSD_{(0.01)}$	0.56		0.51		0.56	
CV (%)	23.72		16.34		13.75	
SE	0.14		0.13		0.14	

*WAI = Weeks after inoculation. Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT.

Appendix IV. Effect of IBA on the number of root in two varieties of Banana

IBA (mg/L)	Number of root					
	Sagar variety			Sabri variety		
	2 WAI*	4 WAI*	6 WAI*	2 WAI*	4 WAI*	6 WAI*
0.0	0.0 ±0.0c	0.0±0.0e	0.40±0.0 e	0.0±0.0c	0.0±0.0d	0.40±0.0 d
0.5	2.00±0.84a	3.20 ±0.45a	5.20 ±0.84a	1.60±0.71ab	2.40±0.45b	2.40±0.84b
1.0	1.60±0.84b	2.80±0.45ab	4.40±0.55ab	2.20±0.55a	2.60±0.45ab	2.60±0.555b
1.5	1.60±0.45a	2.40±0.55bc	3.60±0.55bc	2.20±0.55a	3.40±0.55a	3.40 ±0.55a
2.0	1.20±0.55a	2.00 ±0.0c	2.80±0.84cd	1.60±0.45ab	2.20±0.0bc	2.20±0.84bc
2.5	1.00 ±0.0b	1.20±0.45d	2.00± 0.0d	1.20±0.0 b	1.40±0.45c	1.40 ±0.0c
$LSD_{(0.01)}$	0.82	0.69	1.02	0.79	0.85	1.16
CV (%)	37.74	20.03	19.25	30.49	24.15	21.47
SE	0.208	0.173	0.258	0.200	0.216	0.294

*WAI = Weeks after inoculation. Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT.

Appendix V. Analysis of variance on days to shoot induction in Sagar variety

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Probability
Between	5	704.967	140.993	112.795	0.0000
Within	24	30.000	1.250	-	-
Total	29	734.967	-	-	-

Coefficient of Variation =9.61%

Appendix VI. Analysis of variance on number of shoot of Sagar variety at 2 WAI

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Probability
Between	5	12.267	2.453	18.400	0.0000
Within	24	3.200	0.133	-	-
Total	29	15.467	-	-	-

Coefficient of Variation = 23.81%

Appendix VII. Analysis of variance on number of shoot in Sagar variety at 4 WAI

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Probability
Between	5	39.867	7.973	25.179	0.0000
Within	24	7.600	0.317	-	-
Total	29	47.467	-	-	-

Coefficient of Variation = 22.81%

Appendix VIII. Analysis of variance on number of shoot in Sagar variety at 6 WAI

	Degrees of freedom	Sum of Squares	Mean Square	F-value	Probability
Between	5	111.500	22.300	53.520	0.0000
Within	24	10.000	0.417	-	-
Total	29	121.500	-	-	-

Coefficient of Variation = 18.44%

Appendix IX. Analysis of variance on shoot length in Sagar variety at 2 WAI

	Degrees of freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	21.571	4.314	115.097	0.0000
Within	24	0.900	0.037	-	-
Total	29	22.471	-	-	-

Coefficient of Variation = 6.51%

Appendix X. Analysis of variance on shoot length in Sagar variety at 4 WAI

	Degrees of freedom	Sum of Squares	Mean Square	F-value	Probability
Between	5	39.812	7.962	133.442	0.000
Within	24	1.432	0.060	-	-
Total	29	41.244	-	-	-

Coefficient of Variation = 6.24%

Appendix XI. Analysis of variance on shoot length in Sagar variety at 6 WAI

	Degrees of freedom	Sum of Squares	Mean Square	F-value	Probability
Between	5	105.752	21.150	268.048	0.000
Within	24	1.894	0.079	-	-
Total	29	107.646	-	-	-

Coefficient of Variation = 4.74%

Appendix XII. Analysis of variance on number of leaf of Sagar variety at 2 WAI

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Probability
Between	5	21.501	4.300	23.758	0.0000
Within	24	4.344	0.181	-	-
Total	29	25.845	-	-	-

Coefficient of Variation = 22.28%

Appendix XIII. Analysis of variance on number of leaf in Sagar variety at 4 WAI

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Probability
Between	5	18.452	3.690	30.024	0.0000
Within	24	2.950	0.123	-	-
Total	29	21.402	-	-	-

Coefficient of Variation = 14.87%

Appendix XIV. Analysis of variance on number of leaf in Sagar variety at 6 WAI

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	20.063	4.013	34.190	0.0000
Within	24	2.817	0.117	-	-
Total	29	22.880	-	-	-

Coefficient of Variation = 10.66%

Appendix XV. Analysis of variance on length of leaf in Sagar variety at 2 WAI

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	7.379	1.476	30.552	0.0000
Within	24	1.159	0.048	-	-
Total	29	8.538	-	-	-

Coefficient of Variation = 14.03%

Appendix XVI. Analysis of variance on length of leaf in Sagar variety at 4 WAI

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	9.887	1.977	40.593	0.0000
Within	24	1.169	0.049	-	-
Total	29	11.056	-	-	-

Coefficient of Variation = 9.98%

Appendix XVII. Analysis of variance on length of leaf of Sagar variety at 6 WAI

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	18.121	3.624	73.595	0.0000
Within	24	1.182	0.049	-	-
Total	29	19.303	-	-	-

Coefficient of Variation = 6.82%

Appendix XVIII. Analysis of variance on days to shoot induction in Sabri variety

	Degrees of Freedom	Sum of Squares	Mean square	F-value	Probability
Between	5	1122.967	224.593	117.179	0.0000
Within	24	46.00	1.917	-	-
Total	29	1168.967	-	-	-

Coefficient of Variation = 9.46%

Appendix XIX. Analysis of variance on number of shoot in Sabri variety at 2 WAI

	Degrees of Freedom	Sum of Squares	Mean square	F-value	Probability
Between	5	4.267	0.853	8.533	0.0001
Within	24	2.400	0.100	-	-
Total	29	6.667	-	-	-

Coefficient of Variation = 23.72%

Appendix XX. Analysis of variance on number of shoot in Sabri variety at 4 WAI

	Degrees of Freedom	Sum of Squares	Mean square	F-value	Probability
Between	5	13.367	2.673	32.080	0.0000
Within	24	2.000	0.83	-	-
Total	29	15.367	-	-	-

Coefficient of Variation = 16.34%

Appendix XXI. Analysis of variance on number of shoot in Sabri variety at 6 WAI

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Probability
Between	5	17.900	3.580	35.800	0.0000
Within	24	2.400	0.100	-	-
Total	29	20.300	-	-	-

Coefficient of Variation = 13.75%

Appendix XXII. Analysis of variance on shoot length in Sabri variety at 2 WAI

	Degrees of freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	7.329	1.466	41.291	0.0000
Within	24	0.852	0.035	-	-
Total	29	8.180	-	-	-

Coefficient of Variation = 7.09%

Appendix XXIII. Analysis of variance on shoot length in Sabri variety at 4 WAI

	Degrees of freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	17.994	3.599	132.092	0.000
Within	24	0.654	0.027	-	-
Total	29	50.880	-	-	-

Coefficient of Variation = 4.88%

Appendix XXIV. Analysis of variance on shoot length in Sabri variety at 6 WAI

	Degrees of freedom	Sum of Squares	Mean Square	F-value	Probability
Between	5	54.144	10.829	147.266	0.000
Within	24	1.765	0.074	-	-
Total	29	55.908	-	-	-

Coefficient of Variation = 5.20%

Appendix XXV. Analysis of variance on number of leaf in Sabri variety at 2 WAI

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Probability
Between	5	11.978	2.396	9.055	0.0001
Within	24	6.350	0.265	-	-
Total	29	18.328	-	-	-

Coefficient of Variation = 29.28%

Appendix XXVI. Analysis of variance on number of leaf in Sabri variety at 4 WAI

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Probability
Between	5	20.536	4.107	20.903	0.0000
Within	24	4.716	0.196	-	-
Total	29	25.252	-	-	-

Coefficient of Variation = 18.42%

Appendix XXVII. Analysis of variance on number of leaf in Sabri variety at 6 WAI

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Probability
Between	5	23.495	4.699	21.049	0.0000
Within	24	5.358	0.223		
Total	29	28.853			

Coefficient of Variation = 13.48%

Appendix XXVIII. Analysis of variance on length of leaf in Sabri variety at 2 WAI

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	10.368	2.074	33.648	0.0000
Within	24	1.479	0.062	-	-
Total	29	11.847	-	-	-

Coefficient of Variation = 14.05%

Appendix XXIX. Analysis of variance on length of leaf in Sabri variety at 4 WAI

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	11.426	2.285	18.208	0.0000
Within	24	3.012	0.125	-	-
Total	29	14.437	-	-	-

Coefficient of Variation = 15.68%

Appendix XXX. Analysis of variance on length of leaf in Sabri variety at 6 WAI

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	23.018	4.604	43.774	0.0000
Within	24	2.524	0.105	-	-
Total	29	25.542	-	-	-

Coefficient of Variation = 9.25%

Appendix XXXI. Analysis of variance on number of root at 2 WAI in Sagar variety

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	12.167	2.433	11.231	0.0000
Within	24	5.200	0.217	-	-
Total	29	17.367	-	-	-

Coefficient of Variation = 37.74%

Appendix XXXII. Analysis of variance on number of root at 4 WAI in Sagar variety

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	34.267	6.853	45.689	0.0000
Within	24	3.600	0.150	-	-
Total	29	37.867	-	-	-

Coefficient of Variation = 20.03%

Appendix XXXIII. Analysis of variance on number of root at 6 WAI in Sagar variety

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	86.000	17.200	51.600	0.0000
Within	24	8.000	0.333	-	-
Total	29	94.000	-	-	-

Coefficient of Variation = 19.25%

Appendix XXXIV. Analysis of variance on root length at 2 WAI in Sagar variety

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	16.225	3.245	187.158	0.0000
Within	24	0.416	0.017	-	-
Total	29	16.641	-	-	-

Coefficient of Variation = 8.57%

Appendix XXXV. Analysis of variance on root length at 4 WAI in Sagar variety

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	32.046	6.409	165.590	0.0000
Within	24	0.929	0.039	-	-
Total	29	32.975	-	-	-

Coefficient of Variation = 8.99%

Appendix XXXVI. Analysis of variance on root length at 6 WAI in Sagar variety

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	59.907	11.981	364.417	0.0000
Within	24	0.789	0.033	-	-
Total	29	60.696	-	-	-

Coefficient of Variation = 5.97%

Appendix XXXVII. Analysis of variance on number of root at 2 WAI in Sabri variety

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	16.667	3.333	16.667	0.0000
Within	24	4.800	0.200	-	-
Total	29	21.467	-	-	-

Coefficient of Variation = 30.49%

Appendix XXXVIII. Analysis of variance on number of root at 4WAI of Sabri variety

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	34.400	6.880	29.486	0.0000
Within	24	5.600	0.233	-	-
Total	29	40.000	-	-	-

Coefficient of Variation = 24.15%

Appendix XXXIX. Analysis of variance on number of root at 6 WAI in Sabri variety

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	79.467	15.893	36.677	0.0000
Within	24	10.400	0.433	-	-
Total	29	89.867	-	-	-

Coefficient of Variation = 21.47%

Appendix XL. Analysis of variance on root length at 2 WAI in Sabri variety

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Probability
Between	5	10.887	2.177	248.844	0.0000
Within	24	0.210	0.009	-	-
Total	29	11.097	-	-	-

Coefficient of Variation = 7.01%

Appendix XLI. Analysis of variance on root length at 4 WAI in Sabri variety

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Probability
Between	5	21.939	4.388	477.637	0.0000
Within	24	0.220	0.009	-	-
Total	29	22.160	-	-	-

Coefficient of Variation = 5.06%

Appendix XLII. Analysis of variance on root length at 6 WAI in Sabri variety

	Degrees of freedom	Sum of Squares	Mean Square	F-value	Probability
Between	5	43.961	8.792	168.798	0.0000
Within	24	1.250	0.052	-	-
Total	29	45.211	-	-	-

Coefficient of Variation = 7.95%

Appendix XLIII. Analysis of variance on number of shoot in Sagar variety

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
2	Factor A	2	25.627	12.813	26.3288	0.0000
4	Factor B	4	38.853	9.713	19.9589	0.0000
6	AB	8	30.507	3.813	7.8356	0.0000
-7	Error	60	29.200	0.487	-	-
	Total	74	124.187	-	-	-

Coefficient of Variation: 28.91%

Appendix XLIV. Analysis of variance on shoot length in Sagar variety

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
2	Factor A	2	0.319	0.160	1.224	0.3012
4	Factor B	4	22.648	5.662	43.4429	0.0000
6	AB	8	12.445	1.556	11.9361	0.0000
-7	Error	60	7.820	0.130	-	-
	Total	74	43.232	-	-	-

Coefficient of Variation: 8.26%

Appendix XLV. Analysis of variance on number of leaf in Sagar variety

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
2	Factor A	2	2.197	1.098	6.7204	0.0023
4	Factor B	4	4.919	1.230	7.5236	0.0001
6	AB	8	4.539	0.567	3.4715	0.0024
-7	Error	60	9.807	0.163	-	-
	Total	74	35.376	-	-	-

Coefficient of Variation: 15.76%

Appendix XLVI. Analysis of variance on length of leaf in Sagar variety

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
2	Factor A	2	1.621	0.810	9.8854	0.0002
4	Factor B	4	1.617	0.404	4.9297	0.0017
6	AB	8	7.560	0.945	11.5261	0.0000
-7	Error	60	4.919	0.082	-	-
	Total	74	25.062	-	-	-

Coefficient of Variation: 10.29%

Appendix XLVII. Analysis of variance on number of shoot of Sabri variety

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
2	Factor A	2	9.627	4.813	45.1250	0.0000
4	Factor B	4	5.920	1.480	13.8750	0.0000
6	AB	8	9.440	1.180	11.0625	0.0000
-7	Error	60	6.400	0.107	-	-
	Total	74	31.387	-	-	-

Coefficient of Variation: 14.94%

Appendix XLVIII. Analysis of variance on shoot length of Sabri variety

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
2	Factor A	2	0.758	0.379	1.5924	0.2119
4	Factor B	4	2.506	0.626	2.6304	0.0430
6	AB	8	10.407	1.301	5.4626	0.0000
-7	Error	60	14.288	0.238	-	-
	Total	74	27.959	-	-	-

Coefficient of Variation: 11.87%

Appendix XLIX. Analysis of variance on number of leaf in Sabri variety

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
2	Factor A	2	1.239	0.620	2.4622	0.0938
4	Factor B	4	7.804	1.951	7.7516	0.0000
6	AB	8	6.137	0.767	3.0476	0.0061
-7	Error	60	15.102	0.252	-	-
	Total	74	30.282	-	-	-

Coefficient of Variation: 17.82%

Appendix L. Analysis of variance on length of leaf in Sabri variety

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
2	Factor A	2	3.811	1.906	24.2499	0.0000
4	Factor B	4	0.176	0.044	0.5587	
6	AB	8	5.094	0.637	8.1032	0.0000
-7	Error	60	4.715	0.079	-	-
	Total	74	13.796	-	-	-

Coefficient of Variation: 11.16%

Appendix LI. Analysis of variance on number of root in Sagar variety

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
2	Factor A	2	12.480	6.240	16.1379	0.0000
4	Factor B	4	4.400	1.100	2.8448	0.0316
6	AB	8	31.920	3.990	10.3190	0.0000
-7	Error	60	23.200	0.387	-	-
	Total	74	72.000	-	-	-

Coefficient of Variation: 23.92%

Appendix LII. Analysis of variance on root length in Sagar variety

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
2	Factor A	1	36.479	18.239	133.0773	0.0000
4	Factor B	3	12.805	3.201	23.3576	0.0000
6	AB	3	15.861	1.983	14.4657	0.0000
-7	Error	32	8.223	0.137	-	-
	Total	39	73.369	-	-	-

Coefficient of Variation: 8.31%

Appendix LIII. Analysis of variance on number of root in Sabri variety

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
2	Factor A	2	10.987	5.493	13.7333	0.0000
4	Factor B	4	12.480	3.120	7.8000	0.0000
6	AB	8	24.080	3.010	7.5250	0.0000
-7	Error	60	24.000	0.400	-	-
	Total	74	71.547	-	-	-

Coefficient of Variation: 24.08%

Appendix LIV. Analysis of variance on root length in Sabri variety

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
2	Factor A	2	25.778	12.889	126.6177	0.0000
4	Factor B	4	6.698	1.678	16.4365	0.0000
6	AB	8	18.990	2.374	23.3193	0.0000
-7	Error	60	6.108	0.102	-	-
	Total	74	57.569	-	-	-

Coefficient of Variation: 6.48%