In vitro propagation of Banana (*Musa spp.)* plantlet by Plant Tissue Culture Technique

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MASTEROF SCIENCE

In

HORTICULTURE (FRUIT SCIENCE)

By

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SUPERVISOR :

Dr. Sanjay Singh (22085)

CERTIFICATE

I hereby declare that the Synopsis entitled **"in vitro propagation of banana (*musa* spp.) plantlet by plant tissue culture technique"** is an authentic record of my work and carried out at Lovely Professional University as requirement for the degree of **Master of Science** in the discipline of **Horticulture**, under the guidance of **Dr. Sanjay Singh (22085)**, Assistant Professor, Department of Horticulture, School of Agriculture and no part of this synopsis has been submitted for any other degree and diploma.

Suneetpal Kaur
(Registration No.11716060)
Horticulture (M.Sc)

CERTIFICATE

This is to certify that synopsis titled**. "in vitro propagation of banana (*musa* spp.*)* plantlet by plant tissue culture technique"** submitted in partial fulfilment of the requirement for the award of degree of **Master of Science** in the discipline of **Horticulture,** is a research work carried out by **Suneetpal Kaur (Registration No. 11716060)** under my supervision and that no part of this synopsis has been submitted for any other degree or diploma.

(**Signature of Supervisor**)
Dr. Sanjay Singh
(22085),
Assistant Professor (Horticulture)

(Signature of co-advisor)
Dr. Madhusmita Disri
Assitt. Professor (Horticulture)

DECLARATION

I hereby declare that the project work entitle **"in vitro propagation of banana (*musa* spp.) plantlet by plant tissue culture technique"** is an authentic record of my work carried out at **Lovely Professional University** as requirements of thesis work for the award of degree of Master of Science in **Horticulture (Fruit Science)** under the guidance of **Dr. Sanjay Singh (22085)**, Assistant professor, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India.

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CONTENTS

Introduction

Banana is one of the popular berry fruit which is available to every common person. It is the seedless fruit grown all over the year. Banana (*Musa spp.*) is a large herbaceous perennial monocotyledonous and monocarpic plant. Banana belongs to family *Musaceae* of order Scitamineae. “Apple of Paradise” i.e., Banana governs its antiquity in India sub-continent from the ancient periods of Ramayana (Approx. 2020 BC) and Kautillta’s Arthashastra (300-400 BC). Supportive evidences of its mythological importance can also be seen in painting and sculptures of Ajanta and Ellora (600 BC).

The edible banana is believed to have originated in the hot tropical regions of South East Asia from M. accuminata and M. balbisiana. India is considered to be one of the centres of origin of M. paradisica as M. balbisiana is of Indian origin. It is interwoven in Indian cultural heritage as its plant along with leaves and fruits are auspicious in all the festive occasions, a social function or worship of God. Banana and plantain is wonder berry, forming staple food of millions of people across the globe, providing a more balanced diet than any other fruit or vegetable.

Unripe fruit can be brewed to form beer and wine or processed into sauce, flour, chips, and crisps.Unripen fruit is also a source of amylase and starch. The green bananas which become palatable after cooking, is popularly referred as plantain, and is a staple food in coastal region in India especially in the state of Kerala, while the fresh fruit consumed after ripening is referred to as dessert banana. It is also a dessert fruit for millions, apart from a staple food owing to its rich and easily digestible carbohydrates with a calorific value of 67-137/100 g fruit.

It is a good source of vitamin A (190 IU per 100 g of edible portion) vitamin C (100 mg/ 100g) and fair source of vitamin B and B2. Fruits are also rich source of minerals like magnesium, sodium, potassium, phosphorus and a fair source of calcium and iron. It makes healthy and salt free balanced diet than many fruits. One hectare of banana yields about 37.5

million calories of energy as compared to 2.5 million calories from wheat and has multifarious uses. About 24 banana seach weighing around 100 g could provide the energy requirement (2400 calories per day) of a man (Singh, 2002).

Banana is not only source of food security but it also plays very important role in income source of small holder farmers. Presently, banana is grown in around 150 countries across the world on an area of 4.84 million ha producing 95.6 million tons (FAOSTAT, 2011). It is grown widely in all types of agricultural systems, ranging from small, mixed subsistence gardens to large, multinational commercial plantations.

In India, banana is fourth important food crop in terms of gross value exceeded only by paddy, wheat and milk products. India is the largest banana consumer and producer in the world followed by Brazil, contributing about 15 per cent of the total world production. Banana is fundamentally a tropical product , develop well in temperature 13° C to 38° C with RH administration of 75% to 85% but in India banana yield is being develop in atmosphere running from damp tropical to dry mellow subtropics. Here the banana is grown under creation frameworks and assorted conditions. Tamil Nadu is leading producer of banana followed by Gujarat, Maharashtra, Andhra Pradesh, Madhya Pradesh. These six states contribute about 78% of total production of country.

Banana is generally propagated vegetatively through suckers but traditionally method is laborious time consuming and not very efficient as far as production of homogenous plant concerned (Baneerjee and De Langhe, 1985). Tissue culture-based micro propagation systems are well developed for bananas and, consequently, can be exploited to multiply elite genotypes. As regards yields performance in banana, tissue culture plants have been reported produce 39% higher yield than sword suckers (Pardeep *et al*.. 1992) (Farahani *et al*. 2008). During 1994 there were 14 units engaged in micro-propagation of fruit crop, 12 units are only for banana. All sorts of varieties (dessert, cooking, brewing) are grown throughout Ethiopia. These varieties are not well studied (Seifu Gebre-mariam, 1999).

The concentration of exogenous cytokinin appears to be the main factor affecting multiplication .There are reports on the use of diphenyl urea derivatives in various cell-culture systems including both callus cultures and micro propagation of many woody-plant species (Sarwar *et al*., 1998; Victor *et al*., 1999; Ainsley *et al*., 2001; Joshi *et al.,* 2003; Kadota and Niimi, 2003). However, the use of diphenyl urea derivatives (thidiazuron) in Musa shoot-tip culture is very rare. Diphenyl urea derivatives are used for propagating cultivars; Kibuzi (AAA), Bwara (AAA)and Ndiziwemiti (ABB) (Arinaitwe *et al.,* 2000). Propagation of banana through *in vitro* techniques has been reported byseveral workers using different explants sources and methods (Jalil *et al*., 2003; Madhulatha *et al*., 2004;Strosse *et al*., 2006; Wong et al., 2006; Venkatachalam *et al*., 2006, 2007; Resmi and Nair, 2007; Shirani *et al.,* 2009).

The researchers state that cultivars responded significantly better to BAP in their shoot proliferation and that BAP was more economical than adenine-based cytokinins. Banana shoot-tip cultures were incubated at an optimal growth temperature of 28 ± 2°C in a light cycle for 12-16 h with a photosynthetic photon flux (PPF) of about 60 µE/m2s1 (Al-amin *etal*., 2009). BAP has a marked effect in stimulatingthe growth of auxiliary and adventitious buds and foliardevelopment of shoot tip cultures (Abeyarante and Lathiff,2002; Buah *et al.,* 2010).Effect of benzylaminopurine (BAP) pulsing was done on *in vitro* shoot multiplication of *Musa acuminata* (banana) cv. Berangan(Najmeh Jafari, Rofina Yasmin Othman and Norzulaani Khalid 2011)where as this study again take placeat the Amhara Region Agricultural Research Institute, Tissue Culture Laboratory, Ethiopia from April to June 2012 to investigate the effect of different concentrations of BAP and NAA on virus free plant regeneration and shoot multiplication(Adane Gebeyehu Demissie 2013).

Micro propagation of the crop is also faced with challenges which need to be addressed in order to improve its production. Some of the problems which hinder the success of the crop include oxidative browning of the wounded tissues and low number of shoots produce per

explant. The review highlights the challenges encountered in tissue culture of banana and explores the *in vitro* propagation techniques by using shoot tip cultures of banana as the possibilities to overcome problems(Munguatosha Ngomuo, Emerald Mneney, Patrick A. Ndakidemi 2014).A case study has been conducted in Jalgaon District of Maharashtra that tissue culture banana is more profitable then sucker culture banana for farmers (K.C Bairwa, A.singh, A. Jhajaira, H.singh, B.K. Goyam, M. Lata and N.singh, 2015).

Objective

Banana is generally propagated vegetative through suckers but traditionally method is laborious time consuming and not very efficient as far as production of homogenous plant concerned (Baneerjee and De Langhe, 1985). Tissue culture-based micro propagation systems are well developed for bananas and, consequently, can be exploited to multiply elite genotypes. As regards yields performance in banana, tissue culture plants have been reported produce 39% higher yield than sword suckers (Pardeep *et al*.. 1992) (Farahani *et al*. 2008). During 1994 there were 14 units engaged in micro-propagation of fruit crop, 12 units are only for banana. All sorts of varieties (dessert, cooking, brewing) are grown throughout Ethiopia. These varieties are not well studied (Seifu Gebre-mariam, 1999).

1. To study the effect of growth regulators (BAP and NAA) on propagation of banana explants.
2. To develop standard protocol for *in vitro* rapid propagation of banana.

Review of Literature

Description of tissue culture

Plant Tissue Culture (PTC) has direct impact on present day agriculture and has been the direct cause for face-lift of modern agriculture in developed countries. This has been possible because of enormous supply of desired type of planting/sowing material obtained by mass micro propagation.

Tissue culture, an important area of biotechnology can be used to improve the productivity of planting material through enhanced availability of identified planting stock with desired traits. The idea of cell and tissue culture were put forth by a German Scientist Haberlandt in 1902.

De Fossard (1985) suggested a combination of physical methods (aimed at reducing the size of microbial populations) and chemical methods (killing remaining microbes) leading to aseptic culture plant tissue culture is the science of growing plant cells, tissues or organs isolated from the mother plant, on artificial media. It includes techniques and methods appropriate to research into many botanical disciplines and several practical objectives. Both organized and unorganized growth is possible *in vitro* (George, 1993).

Tissue culture enables plant material to be produced free of contaminating microorganisms, pests, and diseases, because only axenic explants are introduced into culture. Additionally, source materials may be virus-indexed prior to introduction into culture until four decades ago, plant tissue culture was regarded as merely an experimental tool for highly specialized botanical investigation, but with the progress in research, it has now emerged as a dynamic and important field of endeavourer. *In vitro* culture has found its best commercial application in agriculture, horticulture and forestry.

Common practices

Banana is an important fruit crop of the world which is cultivated over an area of more than four million hectares and its annual production is more than seventy million tonnes (FAO, 2006).It is also the premier fruit of Asia and the Pacific. It is the most important fruit of Indonesia, Thailand, Bangladesh, Vietnam, the Philippines, the South Pacific island countries and also India, where recently banana has been surpassing mango, traditionally the dominant fruit.

Banana also occupies an important position in the agricultural economics of Australia, Malaysia, Taiwan, Srilanka and South China. Taiwan and the Philippines derive substantial earnings form their banana export. It is also an important trade and income. The great bulk of bananas produced in our country are traded and consumed in domestic markets. But Unfortunately, over 40% of the countries production is lost due to poor harvesting and handling techniques, inadequate banana market and due to fungal diseases like panama and pests like banana weevil. These diseases and pests make the harvested crop to be of poor quality thus diminishing the returns to farmers.

Many biotic and abiotic factors are responsible for low yield and production of banana. Virus is one of the major problems. The traditional clonal propagation method appears to be unable to supply the increasing demand for disease free and healthy planting materials of banana. The productivity of vegetatively propagated banana and plantain is greatly reduced by virus disease (Lepoivre, 2000). Moreover, 5-10 suckers can be obtained per plant per year which may be of uniform size and virus free.Furthermore, banana production sometimes becomes seriously affected by different diseases (Rahman *et al*., 2004). As a result, banana productivity decreases and the yield become very poor.

To minimize the above mentioned problems, micropropagation could be an alternative for propagation of planting materials for banana. In this method, over a million of plant can be

grown from a small or even a microscopic piece of plant tissue within a year (Mantell *et al.,* 1985). Moreover, the shoot multiplication cycle is very short (2-6 weeks), each cycle resulting in an exponential increase in the number of shoots and plants multiplication can be continued throughout the year irrespective of the season (Razdan, 1993). Meristem culture offers an efficient method for rapid clonal propagation, production of virus free materials and germplasm preservation in plants (Cronauer and Krikorian, 1984a; Hwang *et al.*, 2000 and Helloit *et al.,* 2002).

Banana is generally propagated vegetatively through suckers. But the traditional method is laborious; time consuming and not very efficient as far as production of homogenous plant is concerned (Banaerjee and De Langhe, 1985).

To overcome this problem, production of saplings using *in vitro* culture techniques could be an effective for production of planting materials of bananas. 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).

Even can say that Plants produced through tissue culture are mostly free from pests and diseases (with a few exceptions). There are many further benefits to using tissue culture plants: firstly they are more vigorous, meaning faster growth and higher yields. Secondly, they are more uniform, allowing for better planned marketing; and they can be produced in large quantities in a short period of time, facilitating distribution of both existing and new cultivars. In other words, tissue culture technology can help banana farmers to make the transition from subsistence to income generation.

For the help of multiplication Plant growth regulators are inevitable for *in vitro* regeneration of crop plants in any artificial medium. They play vital role for good growth by there particular characters. 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, 1984).

Banana plantlet propagation by tissue culture in laboratory

W.C. Wong (1986) gives short communication on base of *In vitro* propagation of banana *(Musa* spp.): initiation, proliferation and development of shoot-tip cultureson defined media Using explants with apical domes, a total of 22 banana cultivars were successfully cultured on a modified Murashige and Skoog's medium containing 6-benzylaminopurine (BA) and indolebutyric acid (IBA). Cultivars varied widely in their multiplication rates in response to cytokinins, BA being consistently more effective than kinetin (Kn).

T.R. Ganapathi *et al*.(1992) *work on* Propagation of banana through encapsulated shoot tips. Shoot tips (ca 4 mm) isolated from multiple shoot cultures of banana cv. Basrai were encapsulated in 3% sodium aiginate containing different gel matrices. Use--of white's medium resulted in 100% conversion of encapsulated shoot tips into plantlets.

Lee *et al*. (1997) studied histology of somatic embryoinitiation and organogenesis from rhizome explants of *Musa* spp. The investigation revealed that the majority of somatic embryos showed normal root formation and consisted of highly vacuolated cells in the poorly structured shoot apex.

V. L. Sheela and S. Ramachandran Nair (2001) from College of Agriculture, Vellayani 695 522, Thiruvananthapuram, India shows experiment on the growth , yield and flowering potential of tissue culture banana *(musa* aab cv. Nendran) to compare sucker plants. The growth

rate is faster in the later stages and they recorded an increase of 6.7 % t in height, 11.92 % ingirth and 3.35 leaves more than the plants from suckers. leess variability is shown during the time taken for flowering and record an increase in yield of 25.63% compared to plants from suckers. The attributes responsible for the increase in yield were length of bunch, length and girth of finger and number of fingers.

Peteira *et al*. (2003) determined the genetic variation of 20 banana accessions (including 3 clones obtained by mutagenesis and somaclonal variation) using random amplified polymorphic DNA markers. PCR products were analysed by electrophoresis in 1.5% agarose gel and TBE buffer stained with ethidium bromide. Each genotype was reported taking the most intense bands creating one matrix of binary values analysed by UPGMA method using Jaccard's similarity coefficients.

Aish Muhammad *et al. (*2004*) work on* banana plantlet production through tissue culture in which banana (*Musa* spp.) cv. Basrai was studied. Shoot tips with MS medium having 5.0 mg/l BAP observation is noted. In that he noted 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 sub culturing.

Gubbuk *et al*. (2004) identified banana off types resulting from spontaneous mutations in field and greenhouse grown banana cv. Dwarf Cavendish in Turkey. Mutations were identified based on the occurrence of altered agronomic parameters and via genetic polymorphisms as detected by random amplified polymorphic DNA (RAPD) analysis.

Md. Al-Amin *et al.* (2009) investigate the effect of different concentrations of BAP and NAA on virus free plant regeneration, shoot multiplication and different concentrations of IBA and IAA on in vitro root formation of banana cv. BARI Banana-I. NAA showed highest shoot

proliferation of 0.75, 2.*75* and 6.25 shoots per explant at 10, 20 and 30 DAI. The longest shoot (1.03, 2.45 and 3.38 cm) at 10, 20 and 30 DAI, produced by the treatment combination of 7.5 mg/l BAP + 0.5 mg/l NAA and with same concentrations maximum number of leaves (2.50, 3.25 and 7.00 leaves/explant at 10, 20 and 30 DAI). For root initiation medium supplemented with different levels of IBA (0, 0.5, 1 .0 and 1.50 mg/l) and IAA (0, 0.5 and 1.0 mg/l)and found highest number of roots produced in 0.5 mg/l IAA + 0.5 mg/l IBA.

Asmare Dagnew *et.al*. (2012) A study was carried out at the Tissue Culture Laboratory of Melkassa Agricultural Research Centre, Ethiopian Institute of Agricultural Research (EIAR) to investigate theeffects of different types and concentrations of cytokinins and auxins on shoot initiationand multiplication, and in vitro shoot rooting of three banana varieties using shoot-tipexplants. MS medium supplemented with combinations of BAP and indole-3-acetic acid (IAA) at 3+0.4, 4+0.4 and 3+0.2 mg/l for Dwarf, Giant and Poyo respectively, were best combinations for high rates of shoot proliferation and elongation. Better rooting was obtained when the shoots were cultured on MS medium with 2.12 mg/l α-naphthalene acetic acid (NAA) for Dwarf and Giant while 1.74 mg/l indol-3 butyric acid (IBA) for Poyo .The best growth was recorded for plantlets transplanted on potting media containing a 3:1 ratio (v/v) of sugarcane filter cake and sand. The hardened plants were transferred and well established to the field.

A micro propagation protocol for banana (*Musa spp*.) cv. Agnishwar was established by using shoot tip culture. Shoot tips obtained by removing leaf sheaths from sucker were cultured aseptically in MS (Murashige and Skoog) medium supplemented with different concentrations of cytokines viz. 6-benzylaminopurine (BAP), kinetin (kin), N6 - (2-isopentyl) adenine (2iP) for multiplication of shoot and auxins viz. Indole-3- butyric acid (IBA), a naphthalene acetic acid (NAA) for induction of root. Maximum multiplication (95%) was obtained in MS medium containing 4.0 mg/1 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 (4.9cm) was observed in MS medium having 5.0 mg/l BAP. IBA at a concentration of 1.0 mg/l was found most suitable for rooting of shoot (Sazedur Rahman *et al.*2013).

Mugo, S.W., *et al.*(2013)Represent their work on Factors Influencing Tissue Culture Banana Output and its Impact on Income in Nyamusi Division, Nyamira North District,Kenya. The research gives the differences between tissue culture and non-tissue culture bananas in terms of yields and income generated. The study shows that tissue culture banana grown had relatively higher incomes as compared to conventional banana growers. A systematic sampling procedure was used to get the sample farmers in the two strata that were interviewed. The researcher employed the following tools for data collection: questionnaires, interviews and document analysis.

Shahnawaz Ahmed *et al*.(2014) published research paper about In vitro multiplication of banana (*Musa* spp) cv. Grand Naine. In his research he said Murashige Skoog (MS) medium with BAP 4.00 mg/l and with IAA 2.00 mg/l resulted in more number of cultures in less time. MS medium + BAP 4.00 mg/l + IAA 2.00 mg/l results more multiple shoots. Maximum rooting was obtained on MS medium with IBA 1.00 mg/l and active charcoal 200 mg/l.

M. H. Ferdous *et al.*(2015) work on BAP and IBA pulsing for in vitro multiplication of banana cultivars through shoot-tip culture. Different concentrations of BAP (0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 mg/l) and IBA (0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) was used in MS medium to assess the influence on in vitro shoot regeneration and subsequent root formation of Amritasagar and Sabri banana cultivars. The survival of the plantlets of both cultivars was more than 82% under ex vitro condition. 0.5 mg/l BAP and 0.3 mg/l IBA can be used with MS media for shoot and root formation of Amrita sagar and Sabri banana cultivars through shoot tip culture.

Sumalatha Avvaru (2016) said that Tissue culture is a technique for immunization and separation of tissues in manufactured medium under in vitro condition. It is a gathering of test strategies by utilizing organs, tissues and cell in a simulated medium under in vitro aseptic

environment. Banana is real natural product crop in India as Maharashtra stands first in banana creation and efficiency in India. By utilizing miniaturized scale spread system these plants are refined in labs and this technique is a vegetative proliferation.

A critical review on Micropropagation of Native Cultivars of Banana is given by Kishor, Abhijith, Y. C. 2 and Manjunatha (2017) in which reviewed about Different kinds of cytokinins and auxins have been used for micropropagation of banana cultivars and shoot proliferation rate is significantly affected by type of banana cultivars and their genomic constitution.

Technical Programme of Work

Location of the experiment site:

The experiment has been planned at school of Agriculture laboratory on 2017 of Lovely Professional University situated geographically at 31˚ 22 minutes and 31.81seconds north latitude and 75˚ 23 minutes and 3.02 seconds east longitude with an altitude 252 meters above sea level, which falls under Trans-Gangetic plain region of agro climatic zone of Punjab. The temperature required for the optimum growth of plant tissues during culture generally ranges between 20°C and 30°C.The culture room should have uniform forced-air ventilation, and a humidity range of 20-98% controllable to ±3%. To regulate temperature and humidity, air conditioners in connection with their regulators.

OBSERVATION TO BE RECORDED:

1. Days required for germination.
3. No. of shoots
4. Shoot length
5. No. of leaves
6. Length of leave
7. Shoot fresh weight
8. Root fresh weight
9. Shoot dry weight
10. Root dry weight
11. leaves area
12. No. of roots
13. Root length

Table: Effect of different concentrations of BAP and NAA on multiplication of banana plantlet at different days after inoculation for different parameters.

Treatment		**Number of parameter**		
BAP(mg/l)	**NAA(mg/l)**	**10 DAI**	**20 DAI**	**30 DAI**
	0			
0	0.5			
	1			
	1.5			
2.5	0			
	0.5			
	1			
	1.5			
5	0			
	0.5			
	1			
	1.5			
7.5	0			
	0.5			
	1			
	1.5			
CV%				
LSD value				

Methodology of Research Work

Explant Selection

Fresh sucker of banana are going to be collected from field. These suckers are then going chopped off about 3-5cm length and washed thoroughly under running water for 10-15 cm. The roots and outer tissues of the suckers then removed with the of the shoots knife. A number of outer leaves removed until shoot measured about 1.2-2.0 cm in length and 1.0cm width at the base.

Sterilization of Explant

To kill parasitic spores and growth, suckers absorbed in Bavistin for 18 hours and washed. Again they are plunged into water contain cleanser for 60 minutes and washed. In Laminar Flow Chamber surface sterilizations done with 70% ethyl alchol, suckers are initially cleaned with 0.1% mercuric chloride ($HgCl_3$) and drops of Tween 20 for 15 mints and washed 3 times with refined water**.**

Tissue culture Media Preparation

The media MS (Murashige and skoog, 1962) is to be prepared by dissolving appropriate amount of nutrients and organic supplements. The MS culture media prepared from sucrose plant growth regulators and agar is used as culture media for shoot initiation and multiplication.

Media Autoclave

The jars with media then autoclaved at 1.06kg/cm at 121℃ pressure for 25 mint after adjusting pH to 5.8.-6.2. and let it to set. The Explants then placed on autoclaved MS media under Laminar flow.

Sucker inoculation:

The isolated and surface sterilized explants were collected carefully and inside the laminar air flow cabinet the explants cutting directly inoculated in taken test tube or jars with 20ml of MS medium supplemented with different concentrations of treatment to be given and cover it with aluminium foil.

Incubation

The banana shoot-tip are incubated at an optimum growth temperature of 28 ± 2° C for 16 hours photoperiod provided by cool white fluorescent tubes. For the development of explants aseptic conditions are kept up inside the development room.

Multiplication

Initial sub-culturing is done when the explants produce some shoots in same medium to produce multiple shoots. The regenerated plantlets after developing sufficient root system are ready to transfer in soil.

An experiment was conducted at the Biotechnology Laboratory in Lovely Professional University, Phagwara, Punjab during the year 2017-2018 with title "Studies on propagation in Banana (***Musa spp***) through in vitro culture". Completely randomized design (CRD) has been used with five different treatments and three replications. The detailed of different treatments, material used and procedure are explained in this chapter.

PREPARATION OF PLANT MATERIAL

Suckers of G9 were taken as explants. The suckers were peeled off to the size of 4 cm at the base 5 cm long shoot tip. The excised shoot tip were washed thoroughly under running tap water after that shoot tip sterilized with 70% ethanol for 1 minute, 2% sodium hypochlorite solution for ten minutes and washed 3 to 4 times with distilled water. After sterilization, the

outer layer were removed, 3 cm long shoot tip were excised and trimmed and transfer to the aseptic condition.

PREPARATION OF MEDIA

Murashige and Skoog medium were used for this experiment. A beaker was filled with 800 ml distilled water and MS medium in powdered form was added slowly to it. 30 mg sucrose is added in to it. The pH was maintained at 5.8. Then 8g of agar was added. The media was transferred to a 1 litter of volumetric flask and make up by adding distilled water. Then medium was autoclaved at 15 psi and 121^0 C for 20 minute and culture medium were allowed to cool at room temperature and stored in culture room.

PREPARATION OF PLANT GROWTH REGULATORS:

Preparation of 1 ppm: Take 100 ml volumetric flask and add 100 mg of plant growth regulator. Then add 2-5 ml of organic solvent to dissolve the powder.

Table: Effect of different concentrations of BAP and NAA on multiplication of banana plantlet at different days after inoculation for different parameters.

EXPECTED RESEARCH OUTCOMES

1. Based on experimental findings, the optimum protocol will develop higher and rapid production or multiplication of banana farmer and industries.
2. Student will get the research publication.
3. Student will be aware about current or global advance research methodology.

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