International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 8, Issue 7 -2021

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2021.08.07.007

Micropropagation of *Musa acuminata* using soil as substrate

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Abstract

Soil acts as an engineering medium for life as it provides a medium for plant growth. It is a habitat for soil microorganisms, a regenerating system for organic and nutrient wastes, water quality regulator, atmospheric composition modifier, making it a crucial key for maintaining ecosystem. Plant tissue culture is an efficient technique used in broad range for propagation and other applications worldwide. Though there are few limitations involved in *in vitro* tissue culture, the present research concentrates on micropropagation of *Musa acuminata* using soil as a substrate media. The explants were treated with different concentrations of plant hormones (IAA, IBA, BAP) at different treatment duration (30mins, 1h, and 2h) and placed in a mist chamber. The plants were observed for the shoot and root initiation. The best combination in the present study is IAA, IBA and BAP in 1:1:1 ratio. Treatment for 120mins showed evident regeneration. Though other treatment duration showed positive shoot and root regeneration, it dried at early stage. The plantlet growth was found to be significant at 70th day. Thus, the plantlets propagated through soil can be directly planted as there is no need for hardening and the plants are less prone to infections.

Keywords: Micropropagation, Plant tissue culture, Regeneration, *Musa acuminata*

Introduction

Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. It is widely used to produce clones of a plant in a method known as micropropagation. Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (totipotency). Single cells, plant cells without cell walls (protoplasts), pieces of leaves, stems or roots can often be used to generate a new plant on culture media given the required nutrients and plant hormones(Espinosa-Leal et al., 2018; Kärkönen et al., 2020).

Current studies in plant tissue culture are highly focused on commercial applications such as crop improvement, secondary metabolite production, and various strategies for inducing genetic interference. Production of transgene and its stable expression through plant tissue culture supported by several genetic tools is again the most critical issue discussed nowadays. Incorporation of such genes produces stress tolerant plants with improved secondary metabolite production(Smeda and Weller, 1991). But, not all plants can be successfully tissue cultured, often because the proper medium for growth is not known or the plants produce secondary metabolic chemicals that stunt or kill the explant. Some plants are very difficult to disinfect of fungal organisms(Jaskulak and Grobelak, 2017).

Soil acts as an engineering medium, a habitat for soil organisms, a recycling system for nutrients and organic wastes, a regulator of water quality, a modifier of atmospheric composition, and a medium for plant growth, making it a critically important provider of ecosystem services. Soil is a major source of nutrients needed by plants for growth (Passioura, 2002). The three main nutrients are nitrogen (N), phosphorus (P) and potassium (K). Together they make up the trio known as NPK. Other important nutrients are calcium, magnesium and sulfur. Plants also need small quantities of iron, manganese, zinc, copper, boron and molybdenum, known as trace elements because only traces are needed by the plant(Gregory and Nortcliff, 2013).

Banana (*Musa* sp) is one of the most important fruit crops grown in India. In respect of area it ranks second and first in production only after mango in this country. India leads the world in banana production with an annual output of about 16.820mt. There are various types of banana cultivars like Dwarf Cavendish, Robusta, Poovan, Nendran, Red Banana, Ney Poovan, Virupakashi, Pachanadan, Monthan, Karpuravalli, Safed Velchi Musa, etc., Fertility of soil is very important for successful cultivation, as banana is a heavy feeder. Banana is one of the few fruits, which has a restricted root zone. Hence, depth and drainage are the two most important considerations in selecting the soil for banana(Imam and Akter, 2011; Swathi et al., 2011).

Therefore, the present research is a study on micropropagation of *Musa acuminata* using soil as a substrate media. The explants were treated with different concentrations of plant hormones (IAA, IBA, BAP) at different treatment duration (30mins, 1h, and 2h) and placed in a moist chamber. The plants were observed for the shoot and root initiation

Materials and Methods

Collection and preparation of explants

The sample was collected from the good yielding banana grove – *Musa acuminata* (poovan). Shafts were sliced into small pieces of size 1cm and washed thoroughly using distilled water. The explants were dispersed in 2% Activated charcoal for 2 hours for removal of phenolic compounds. Then the explants were treated with 0.1% bavistin solution for 20mins and washed with distilled water.

Treatment of explants with growth hormones

Indole acetic acid (IAA), Indole 3-butaric acid (IBA), Benzyl amino purine (BAP), were the hormones used for treatment of explants. Three concentration (3mg, 6mg & 9mg) of hormones were used at three treatment period (30mins, 1h & 2h). Table-1 shows the combination of hormones used for treatment.

Table-1: Combination of hormones used for treatment

S.No	Combination	Concentration	Duration
1	Control	-	-
2	IBA	3mg	30mins
3	BAP	3mg	30mins
4	IAA	3mg	30mins
5	IAA + IBA +BAP	3mg	30mins
6	IBA	6mg	30mins
7	BAP	6mg	30mins
8	IAA	6mg	30mins
9	IAA + IBA +BAP	6mg	30mins
10	IBA	9mg	30mins
11	BAP	9mg	30mins
12	IAA	9mg	30mins
13	IAA + IBA +BAP	9mg	30mins
14	IBA	3mg	60mins
15	BAP	3mg	60mins
16	IAA	3mg	60mins
17	IAA + IBA +BAP	3mg	60mins
18	IBA	6mg	60mins
19	BAP	6mg	60mins
20	IAA	6mg	60mins
21	IAA + IBA +BAP	6mg	60mins
22	IBA	9mg	60mins
23	BAP	9mg	60mins
24	IAA	9mg	60mins
25	IAA + IBA +BAP	9mg	60mins
26	IBA	3mg	120mins
27	BAP	3mg	120mins
28	IAA	3mg	120mins
29	IAA + IBA +BAP	3mg	120mins
30	IBA	6mg	120mins
31	BAP	6mg	120mins
32	IAA	6mg	120mins
33	IAA + IBA +BAP	6mg	120mins
34	IBA	9mg	120mins
35	BAP	9mg	120mins
36	IAA	9mg	120mins
37	IAA + IBA +BAP	9mg	120mins

Inoculation of explants in nutrient soil

Nutrient soil was prepared by mixing equal volumes of vermicompost, cocopeat, sand, red soil and manure (1:1:1:1). The mixture was sterilized using autoclave at 121°C for 15mins. After sterilization, the soil was filled in a tray and explants were inoculated on it after the hormone treatment.

Construction of mist chamber and incubation

Essential Components:

1. Structure: Galvanized iron frame Structure
2. Cladding: UV Stabilized Polyfilm / Poly carbonate Sheet
3. Forced Air Cooling System: Corrugated Cellulose Pad and Axial Flow Flan
4. Fogging: Overhead fogging system at the gutter height of mist chamber

5. Irrigation: Knapsack sprayer/ Water & Fertilizer applicator boom irrigation system
6. Alluminate Screen: to cut off the excess the sunlight in order to maintain the temperature inside the mist chamber
7. Shade Net: Over the top of the mist chamber probably 70:30 will be preferred for the peak summers
8. Root trainer: for sowing the seedlings
9. Inert Martial: Coco peat, Perlite, Vermiculite

Relative humidity is maintained at high level (95 %) with the help of mister's, which spray water under high pressure. Size of mist partials lies between 50 to 100µm. Figure shows mist chamber used in the study. The explant containing tray was placed inside the mist chamber and incubated. The initiation of shoot and root was observed at weekly time intervals.

Results

Table-2: Effect of plant hormones and treatment duration in regeneration

S.No	Combination	Duration (mins)	Concentration (mg)		
			3	6	9
1	IBA	30	-	-	-
2	BAP		+	-	-
3	IAA		-	+	-
4	IAA + IBA +BAP		-	-	-
5	IBA	60	-	+	-
1	BAP		-	-	-
2	IAA		-	-	-
3	IAA + IBA +BAP		-	-	+
4	IBA	120	-	-	+
5	BAP		+	-	-
1	IAA		-	-	-
2	IAA + IBA +BAP		++	++	++

'-' denotes no initiation; '+' denotes initiation of shoot and root but dried at early stage; '++' denotes initiation and defined growth

Plant regeneration was observed on different hormone combination treatments and treatment duration. Control plants showed no regeneration. Among all the hormone treatment the combination of IAA, IBA and BAP at 1:1:1 showed significant plant regeneration (Table-2). From the three treatment periods, 120mins showed better results than the other periods.

Best combination of hormones (IAA + IBA +BAP) showed increased growth on 21days, 49 days and 70 days (Figure-1). At 3mg treatment the shoot and root length were found to be 1.5cm and 0.3cm at 21st day; 2.8cm and 1.1cm at 30th day; and 5cm and 2.4cm at 70th day. At 6mg treatment the shoot and root length were found to be 2.2cm and 0.7cm at 21st day; 4.7cm and 1.8cm at 30th day; and 6.2cm and 2.9cm at 70th day. At 9mg treatment the shoot and root length were found to be 3.1cm and 1.2cm at 21st day; 6.4cm and 2.3cm at 30th day; and 8.2cm and 5.1cm at 70th day. 9mg treatment showed higher shoot and root renegenration (Figure-2 & 3).

Figure-1: Shoot and root development

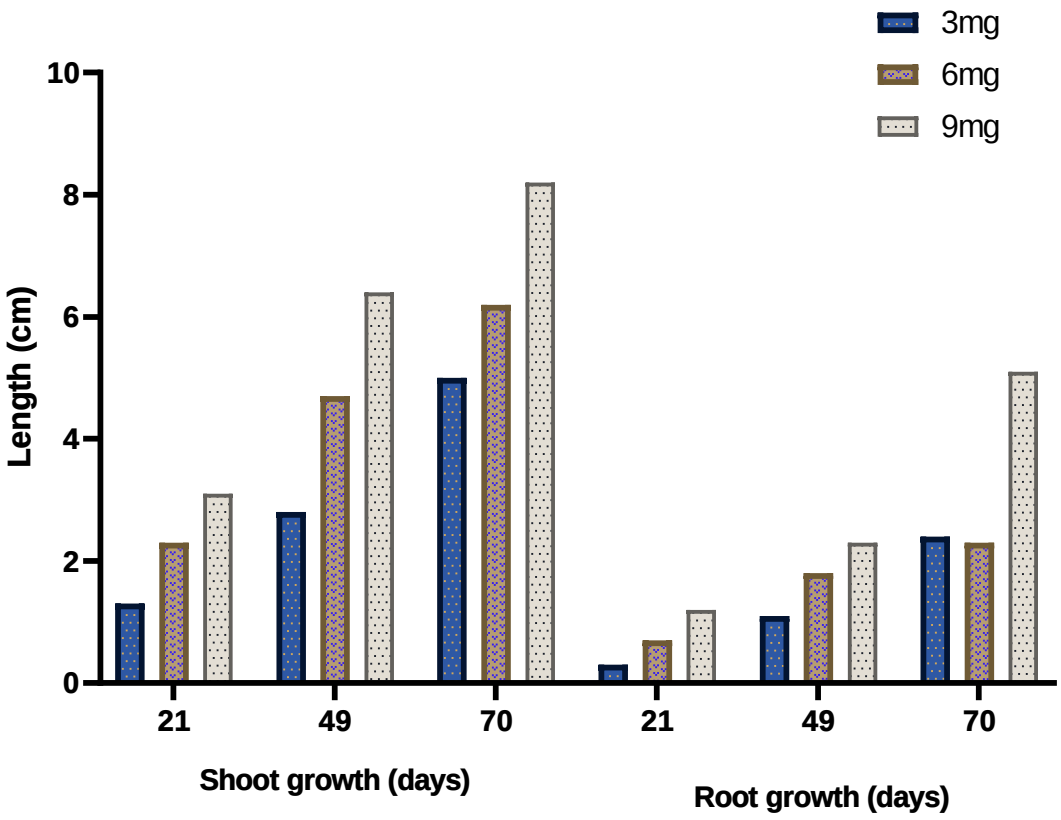

Figure-2: 9mg hormone combination treated plant after 30days

Figure-3: 9mg hormone combination treated plant after 70days

Discussion

As there are several disadvantages on plant tissue culture like: Plants are highly susceptible to diseases, all the plants of the stock will share this undesirable trait and be susceptible to particular diseases. If precautions are not taken, the whole stock may be contaminated or infected. The major disadvantage is it requires more labour and cost more money (Hahne, 1991). To overcome these consequences, the present study is a preliminary effort on regeneration of plants using soil as a substrate.

In this study, different combinations of hormones are used. The best treatment was found to be the combination of IAA, IBA and BAP altogether. Indole-3-acetic acid (IAA) is the main auxin in plants, controlling many important physiological processes including cell enlargement and division, tissue differentiation, and responses to light and gravity. It is now generally agreed that indole-3-acetic acid (IAA) is the major and most abundant auxin in plants. IAA plays a key role in the regulation of plant growth and development. Over the last few years significant progress has been made in understanding the IAA-induced signal transduction pathway(Shahab et al., 2009; Yamini et al., 2021).

Indole-3-butyric acid (IBA) is a plant growth auxin that enhances the development of plants and food crops when it is applied to cuttings, leaves, or soil. It is been proven to increase flower production and crop yield. It is used primarily to enhance plant growth. Although the exact method of how IBA works is still largely unknown, genetic evidence has been found that IBA may be converted into IAA through a similar process to β-oxidation of fatty acids(Frick and Strader, 2018; Ludwig-Müller, 2000).

6-Benzylaminopurine, benzyl adenine, BAP or BA is a first-generation synthetic cytokinin that elicits plant growth, development responses and stimulating fruit richness by stimulating cell division. It is an inhibitor of respiratory kinase in plants, and increases post-harvest life of green vegetables. Influence of cytokinin as 6-benzylaminopurine (BAP) in combination with other methods on postharvest green color retention on broccoli heads and asparagus spears, showed positive results for quality retention.

Adenine-based cytokinins are used in several Musa spp. for in-vitro propagation. N6-benzylaminopurine (BAP) is the most commonly preferred cytokinin. The others are isopentyladenine (2-ip), zeatin and kinetin. The concentration of exogenous cytokinin appears to be the main factor affecting multiplication. Many other studies have reported the use of auxins and cytokinin in tissue culture. Gubbuk and Pekmzci (2004) reported that moderate concentrations of cytokinins increased the shoot proliferation rate, but very high concentrations decreased multiplication and especially depressed shoot elongation. In another study, Buah et al. demonstrated that differences exist in the relative strengths of different cytokinin types in inducing shoots. This differential ability of different hormones in inducing shoots in vitro may be attributed to factors such as stability, mobility and the rate of conjugation and oxidation of hormones (Ayoola-Oresanya et al., 2021; Rahman et al., 2013).

The concentration and combination of auxins and cytokinins in the nutrient mediums is an important factor which determines successful plant regeneration. As stated above the best combination in the present study is IAA, IBA and BAP in 1:1:1 ratio. The plantlet regeneration was found to be significant at 70^{th} day. The plantlets can be directly planted on soil as there is no need for hardening of plants (as performed on conventional tissue culture method) and the plants are less prone to infections.

Conclusion

The explants of *Musa acuminate* were treated with different concentrations of plant hormones (IAA, IBA, BAP) at different treatment duration (30mins, 1h, and 2h) and set down in a moist chamber. The plants were observed for the shoot and root initiation**.** The best combination in the present study is IAA, IBA and BAP in 1:1:1 ratio. Though other treatment duration showed positive shoot and root regeneration, it dried at early stage. Treatment for 120 mins showed significant plant growth. The plantlet growth was found to be higher at 70^{th} day. The plantlets can be directly planted on soil as there is no need for hardening of plants and the plants are less prone to infections.

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Quick Response Code

DOI:10.22192/ijarbs.2021.08.07.007

How to cite this article:

Visvanand R, Rekha Shanmugam, Sanjay Prasad S, Karkuvelraja R, Midhila TS, Vijay R , Yoganand R. (2021). Micropropagation of *Musa acuminata* using soil as substrate. Int. J. Adv. Res. Biol. Sci. 8(7): 56-63. DOI: http://dx.doi.org/10.22192/ijarbs.2021.08.07.007