In-Vitro Response of Ornamental Banana (Musa Spp.)

Dayarani .M, Dr. Dhanarajan.M.S, and Dr.Uma.S

***Abstract*—**Banana (Musa spp.) belongs to the family Musaceae. *Musa laterita* is a related wild species of Banana which belongs to Rhodochlamys section. It is known as ornamental banan because of its bright brick red coloured bracts. Objective of this work is to develop highly efficient and reproducible in vitro shoot tip multiplication protocols. Mercuric chloride and Sodium hypochlorite (5.25%) were used in two different concentrations each, out of which 0.25% of mercuric chloride proved best in decontaminating the explants. .Among the different hormonal combinations tried, use of 3mg/l BAP along with 1mg/l IAA was found suitable for in-vitro multiplication of *Musa laterita*.

***Keywords*—** Banana, BAP, IAA, In-vitro.

I. Introduction

BANANA (Musa spp.) is the ancient and most valued fruit crop of the mankind. It belongs to the family Musaceae and section Eumusa. In India, it is the most important fruit crop, occupying fourth place among the commodities. It is not only used as fruit and vegetable , also it finds its use in all religious functions. Without the banana plan, no function is complete. But the crop is severely affected by many viral and bacterial diseases and also with many insects/pests. As it is a long duration crop ,improvement in this crop also takes a long time. Hence, using a ready source of resistant genes will ease the breeding programmes. There are certain species of Banana which harbour resistant genes for biotic and abiotic stresses. They are also important from ornamental point of view. They belong to the section Rhodochlamys of Eumusa. These species of Banana are seen growing wild in regions of North Eastern parts of the country, Myanmar etc.

Musa laterita is one of the species under Rhodochlamys section known for its bright brick red coloured bracts.(Fig.1). Its distribution is mostly from sea level up to an altitude of only 150 m above MSL. It is frequent in home-gardens of Karnataka, Assam and Meghalaya states, as ornamental plants. To commercialize these plants as ornamentals or to use it in breeding programmes, in-vitro propagation is a preferred method. Shoot tip culture of bananas provides excellent advantages over traditional propagation, including high multiplication rate, physiological uniformity, the availability of disease-free material all the year round, rapid dissemination of new plant materials throughout the world, uniformity of shoots, short harvest interval in comparison with conventional plants, and faster growth in the early growing stages compared to conventional materials. It also plays a vital role in the distribution of germplasm, conservation and safe exchange of planting material. Decontamination procedure and plant growth regulators are the two important factors to be considered to develop a standard tissue culture protocol. Hence in the present work, these factors are studied in *Musa laterita* to develop highly efficient and reproducible in vitro shoot tip multiplication protocols, followed by efficient rooting.

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II. Materials And Methods

The planting materials of *Musa laterita* were collected from field gene bank of National Research Center for Banana,Trichy. The suckers of three months of age, grown under field conditions were carefully extracted, without damaging the rhizome portion and was brought to the preparation room of the lab. 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 leaf sheaths were removed until the shoot measured about 5.0 cm in length and 3.0 cm width at the base,washed in 0.1% cetrimide. Again the suckers were cut to a size of 2.0cm x 2.0cm x 2.0cm treated with 0.1% cetrimide for 10 minutes followed by different concentrations of mercuric chloride or sodium hypochlorite(5.25%) and a few drops of Tween 20. After removing from the solution, 3-4 washes were given with sterile distilled water to remove traces of disinfectant. Finally shoot tips containing meristem along with 6 to 8 leaf sheaths were excised aseptically and immediately placed in semi solid MS culture media [1]. This medium was supplemented with different concentration of plant growth regulators to obtain maximum morphogenic response. Five different concentrations of BAP were used (1mg/l, 2mg/l, 3mg/l, 4mg/l and 5mg/l).Response was studied by using BAP alone and also in combination with Indole Acetic Acid(IAA). Ascorbic acid (10mg/l) was used in the medium as antioxidant to reduce blackening. pH of the medium was adjusted to 5.8 before autoclaving. The culture bottles were incubated at 26°C with an RH of 60 to 70 per

cent and 16 hr light cycle measuring 2000 to 3000 lux .

Established cultures were regularly transferred every 3 weeks by subdividing shoot clusters with a scalpel. The explants were sub-cultured 3 times and after each sub-culture, the shoots per explants were counted and shoot lengths were measured. In the propagation stage, shoots per explant and average shoot length were recorded. In the rooting stage, plant height, root numbers, average root length and stem diameter (determined by measuring the diameter above 1 cm of pseudostem) were measured.

Fig.1 *Musa laterita* plant

Fig. 2 In-vitro plants of *Musa laterita* (3 mg/l BAP)

III. Results and Discussion

Mercuric chloride and Sodium hypochlorite (5.25%) were used in two different concentrations each.(Table-1). When mercuric chloride was used as disinfectant, both percent contamination and explant survival decreased with increased concentration of mercuric chloride. Same trend was observed with Sodium hypochlorite also. Though a series of washes are given to the explants after surface sterilization, effect of disinfectant is exhibited on various growth parameters. One such parameter is days taken for greening which is nothing but activation of apical or axillary buds. Days taken for greening was minimum with 50% of sodium hypochlorite(5.53 days) but use of 0.25% mercuric chloride took 6.33 days for greening which is not significantly high. So with a highest survival of 90% and medium number of days taken for greening, use of 0.25% mercuric chloride for 5 minutes could be successfully utilized for surface sterilization. Though sodium hypochlorite is a good disinfectant and survival per cent was high, contamination rate was also high. Hence, sodium hypochlorite is not a preferred disinfectant. This may be because of long time of exposure of 30 min in sodium hypochlorite.

In tissue culture, plant growth regulators are critical media components in determining the developmental pathway of the plant cells. Cytokinins are generally known to reduce the apical meristem dominance and induce both axillary and adventitious shoots formation from meristematic explants in banana [2]. 6-Benzyl Amino Purine (BAP) is the most commonly preferred and well suited cytokinin for the in-vitro propagation of banana [3],[4]. The effectiveness of BAP over other cytokinins in inducing multiplication of shoot tip cultures has been reported in different cultivars of bananas[5][6][7][8]. BAP has a marked effect in stimulating the growth of axillary and adventitious buds and foliar development of shoot tip cultures [8]. Also, 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 [6].Hence, BAP was used in varied concentrations for shoot tip culture of *Musa laterita*. viz., 1mg/l, 2mg/l, 3mg/l, 4mg/l and 5mg/l. IAA was tried in single concentration of 1mg/l. The results are shown in Table II.

TABLE I
EFFECT OF MERCURIC CHLORIDE ON SURFACE STERILIZATION

Disinfectant (%)	Duration	Contamination (%)	Survival (%)	Days for greening
Mer.Chloride 0.25	5 min	76.5	90.0	6.33
0.50	**5 min**	**68.3**	85.3	9.96
Sod.hypochlor 50	30 min	79.3	87.4	5.53
100	30 min	77.6	86.3	7.78

TABLE II
SHOOT PROLIFERATION RESPONSES TO PLANT GROWTH REGULATORS IN *MUSA LATERITA*.

Treatment mg/l	No. of buds on 20th day	No. of buds on 40th day (1st subculture)	No. of buds on 60th day (2nd subculture)
Control (MS)	0.0	0.42	0.63
BAP(1.0)	0.0	0.71	0.91
BAP(2.0)	0.61	1.02	2.14
BAP(3.0)	1.33	3.47	4.21
BAP(4.0)	1.81	2.84	3.16
BAP(5.0)	0.0	2.32	2.68
BAP(1.0)+ IAA(1.0)	0.0	0.69	0.89
BAP(2.0)+ IAA(1.0)	0.69	1.04	2.19
BAP(3.0)+ IAA(1.0)	1.40	3.52	4.62
BAP(4.0)+ IAA(1.0)	1.82	2.86	3.21
BAP(5.0)+ IAA(1.0)	0.0	2.33	2.52

On an average, 4mg/l BAP exhibited more bud induction followed by 3mg/l with 1.33 buds on 20th day after inoculation. But on 40th day, i.e, during the end of 1st subculture, 3mg/l BAP induced higher bud production with 3.47 buds followed by 4 mg/l BAP(2.84 buds).On 60thday i.e, by the end of second subculture, 3mg/l BAP continued to exhibit higher bud production resulting in multiple shoot formation, which showed the same trend in later subcultures also(Fig.3) In combination with IAA also, almost a similar trend was obtained. Whereas use of 5mg/l of BAP gave a different result showing reduction in the number of buds. Venkatachalam et al reported a reduction in the number as well as length of shoot that occurred with exposure to high levels of BAP alone (44.44 µM) in banana cv. Nanjanagudu Rasabale [9]. D'Amato reported that application of high concentrations of growth hormones for clonal propagation is often disadvantageous since they may cause various chromosomal abnormalities resulting in the production of non true-to-type plants.[10].The results indicate that use of 3mg/l BAP along with 1mg/l IAA is suitable for in-vitro multiplication of Musa laterita.

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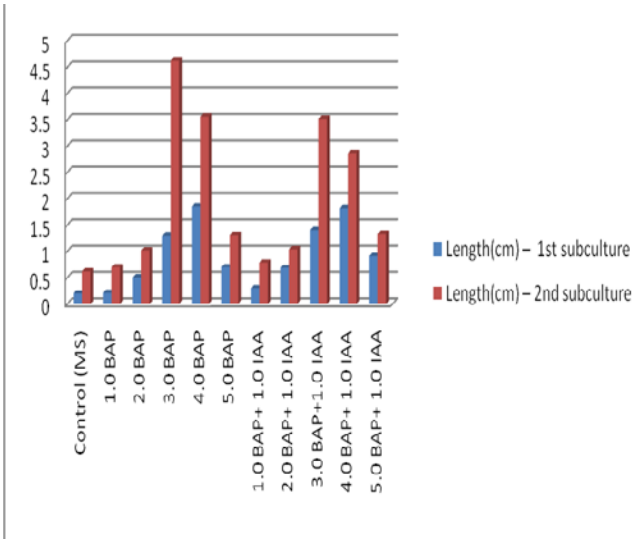

Fig. 1: Graph Showing Effect Of Bap On Shoot Length In Musa Laterita

Shoot length showed gradual increase with increase in BAP levels up to 4mg/l, then showed a decreasing trend. Similar trend was observed when IAA was used. (Fig. 1). Amount of BAP in the media influences the growth of plants. BAP as an antagonistic effect at higher concentrations.

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