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Evaluate Standardize Specific Media For Micro Propagation Of Banana

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ABSTRACT
Among all fruit crops banana is one of the important fruit crop and important source of food for millions of people form many decades. Here we study the explants (i.e. Suckers) of two banana cultivars were cultured on Murashige and Skoog (MS) medium supplemented with altered concentrations of BAP and Kinetin sole and in combination for shoot commencement and proliferation. For testing, BAP concentrationwas (0.5mg/l and 1.0mg/l) and kinetin (0.5mg/l and 1.0mg/l). Whereasfor shoot commencement the combination 0.5mg/l BAP+0.5mg/l kin and 1.0mg/l BAP +0.5mg/l kin. For proliferation, concentration of BAP(2.0,2.5 and 2.5mg/l) alone and in combination BAP+ Kin (1.5mg/l +1.5mg/l, 2.0mg/l +2.0mg/l and 2.5mg/l +2.5mg/l) were used. The rapid shoot commencement obtained from MS medium improved with the combination of 1.0mg/l BAP with 0.5mg/l kin (8 and 10 days) in both Grade nine and Giant Cavendish respectively. The highest proliferation shoot (6.0 and 4.5/explants), in Grade nine and Giant Cavendish were observed on the MS medium fortified with 2.5mg/ l BAP+2.5mg/l kin and 2.0mg/l BAP +2.0mg/l Kin respectively. For root induction 1.5mg/l BAP and 1.5mg/l IAA each tested separately on MS medium. The best performance is showed by BAPwith 5.12 and 4.69 root/ plantlet after four weeks of inoculation in Grade nine and Giant Cavendish respectively. Vitro plantlets were transferredto green house after 12 weeks for acclimatization where 82% and 88% survival rate was recorded in Grade nine and Giant Cavendish respectively. The study reveals that of two cultivars showsdifferences in shoot commencement, shoot proliferation and rooting. Grade nine found to be more responsive and had highest rate shoot commencement, shoot proliferation of for in vitro techniques among the two cultivars.
Keyword:*Banana; BAP; IAA;MS Media*

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INTRODUCTION

Bananas and plantains (*Musa app.*) are grown in more than 125 countries. Primarily in tropical countries, throughout the world. They are grown in a range of environmental conditions with relatively minimum expenses for cultivation and produce fruit year round. They are amongst the 29 crop species on which people largely depend for most of their calories and proteins [1]**.**They are propagated vegetative as majority is seedless hybrid varieties. In India it is grown in 3,96,000 hectare , mainly in Tamil Nadu , West Bengal, Kerala , Maharashtra , Gujarat , Karnataka North Eastern state etc., with a production of 10.4 million tons per year [3]. Some of 50% of its world production is in Africa, 25 % in the South America, 15% in Asia 10% in Central America [7]**.** The development of breeding techniques and propagation practices has launched quite a revolution in the field of banana cultivation during the last fifty years.

Banana is rich in carbohydrate, Vitamin B, C and A minerals such as potassium,calcium, magnesium and sodium. It is nutritious, palatable and easily digestible and remains available throughout theyear. This crop forms the fruit diet of more than 400 million people, ranks fourth in the category of staple food products, after rice, wheat, and milk [2]**.**Micro propagation and in vitro germination of hybrid have played a key role in plantain and banana improvement programs worldwide [6, 8]**.**

Banana and plantain (*Musa* sp.) is a cropof tremendous economic socialimportance in the humid and sub humidtropical region of the world [5].Micro propagation or in-vitro techniqueswere established for fast multiplication of bananas [9]**.**

MATERIAL AND METHODS

Research work was conducted at Tissue Culture Laboratory of Department of Plant Molecular Biology and Genetic Engineering, Sam Higginbottom University of Agriculture and Technology, Prayagraj during 2016-2018.

Glassware and is sterilization

All the glassware was cleaned and or rinsed with chromic acid, first and then washed thoroughly with running tap water and distilled water, Hot air oven dried and used. Borosil glassware used comprised of 25x150mm test tube, culture bottle (130 mm height, 5.5cm circumference with bottle volume 300 ml), pipettes of various capacities, measuring cylinder and beakers etc. All the pipettes, measuring cylinders and beaker were used after rinsing first with tap water than with distilled water.All glassware were sterilized either in autoclaved at 15 psi for 20 min, or sterilized in hot air oven at 160-1800C and after wrapping in brown paper. The sterilized glassware was cooled down at temperature before use.

Growth hormones

Stock solutions of all chemicals were prepared from Analar grade chemicals, manufactured by BDH, Hi-Media, Merck, GlaxoSmithKline Qualigen and Sigma chemicals, USA. Agar-agar used was of bacteriological grade manufactured by BDH/ Sigma chemicals. Sucrose used was of lab Grade of BDH/ HI media make.

The stock solutions of cytokine BAP or kinetin was prepared by dissolving 50 mg of chemical by slight heating and dissolving in few drops of 0.1N HCl or 0.1N NaOH and then making the volume with distilled water to 50 ml to give a final concentration of 0.1 mg/ml. Similarly, stock solution of 1mg/ml of IAA (Indole-3 Acetic acid) was prepared by dissolving known quantity of chemical in 1-2 ml absolute alcohol and making up the final volume with distilled water. All the stock solutions of growth hormones vizauxin and cytokinin were freshly prepared and stored at 40C in refrigerator. Iron source was used in chelating form as FeSO4.7H2O-EDTA with concentration of 27.3 and 37.8 mg/l. Stock solution was autoclaved at 1210C and 15 PSI and stored in Amber colour bottle in refrigerator at 40C for further application.

Culture media

Murashige and Skoog medium (1962) was used in the present study with certain modification for shoot induction, growth, shoot multiplication and hardening of tissue culture raised banana plants.

RESULT AND DISCUSSION

Sterilization of explants

Table no 1 shows composition of MS media MSB0 Suckers were cut and reduced in shows size by removing the external leaf sheath and trimming rhizome to about 5 x 3 cm, keeping the centrally placed shoot tip intact. Explants were further trimmed to 1.5 x 1 cm before sterilization. All the explants were sterilized under aseptic conditions and transferred to culture media MSB0. This cone like cream color structure of explants contained 3 to 4 leaf sheaths with centrally placed shoot tip inside. Explants turned brown if stored for longer time at room temperature.

The sterilization of explants carried out using 70% alcohol, and mercuric chloride (0.1%). Treatment of explants with 70% absolute alcohol was not effective and explants remained contaminated and therefore discarded. Shoot tip explants were sterilized using mercuric chloride at different concentrations and for different time intervals. It was observed that most of contaminants appeared during first week of inoculation of explants to the culture

medium, area of which increased later with the lapse of time. Initially, contamination was confined around the explants and then it spreads on either surface of culture medium and in deeper layers of explants as well as on culture medium both. The external surfaces of all the tissues including control explants were also damaged and its surface also turned light brown to black with the lapse of time. Those explants that showed growth, the outer most layers of tissue irrespective of rhizome or leaf sheath appeared externally damaged and turned brown while inner layers of tissue remained live and white cream in color. The explants that were dead and turned brown to black without any growth, exhibited total browning even in deeper layers of explants. Some of the cultures were observed contaminated even after 4 weeks of culture and during subculture period pointed out the presence of systemic infection in the tissue.

To check in situ infection a pre-treatment with bavistin (1.0%) corbendazim fungicides 1gm and almox 10 mg for a period of one hour was also given before sterilization.

The blackening of the rhizome tissue was concentration and time dependent i.e. it increased with concentration of sterilizing agent and time period of sterilization. The browning / blackening also increased with increase in the time of incubation in culture medium.

Mercuric chloride was found effective in removing contamination from banana explants. Exposure of shoot tips explants to 0.1% mercuric chloride for 8 min. gave satisfactory removal of bacterial as well fungal contamination up 7-10 to 100% of the explants inoculated. With the increase in concentration of mercuric chloride from 0.1 to 0.2% and time of treatment from 8-10min, blackening percentage of explants increased to 100% and necrosis of the shoot tip without any growth. At higher concentration and long exposure of sterility all the explants turned brown to black and were dead. When shoot tip explants were sterilized with 0.2% mercuric chloride for 6 min, it was observed that 100% of explants survived without any contamination. At this concentration, explants showed visible growth and blackening as well. Maximum survival percentage of 100% was achieved when shoots tip of explants were treated with 0.1% of mercuric chloride for 8 min or 0.2% of mercuric chloride for a period of 6 min. However, it was also noted that those explants that were treated with 0.1% of mercuric chloride for 8 min. performed well during subculture.

Since the infection was also observed during the subculture and after 4 weeks, an attempt was made to remove infection from shoot tip explants by pre-treatment with bavistin (1.0%) for one hour and subsequent sterilization with mercuric chloride (0.1%) for 8 min. further improved the survival percent to 100% contamination. From the results, it is concluded that pretreatment of explants with bavistin (1.0%) for a period of one hour before sterilization with thorough washing in running tap water and surface sterilization with mercuric chloride (0.1%) for 8 min considerably reduced the rate of infection in explants during culture and even at later stages of growth bavistin was helpful in removing in situ contamination to a great extent. This method of pretreatment with bavistin and mercuric chloride described above was used for sterilization of explants in present study for in vitro culture of banana shoot tips. Similar result found by **Kelta *et al.*, [4].**

Table no 1: Composition of MS media MSB0

Medium no	BAP Mg/l	IAA Mg/l	Kinetin Mg/l	pH	No of plant	Growth of plant	Death of plant	Day	Treatment
MSB_0	00	00	00	5.4	8	00	5	**7-10**	Bavistin 1gm/l corbendazim fungicide 1gm/l almox 10mg/l

Table no 2 shows composition of MS MSB1 BAP 1mg/l and MSB2 BAP 2mg/l IAA Kinetin amount 0 adjusted the pH 5.8 made up the media used of 72 hours. Selected the 5 suckers treatedwith bavistin 1gm/l corbendazim fungicide 1gm/l and almox 10 mg/l mixture the water room temperature one hour. After washing with the running tap water. The suckers were cut to the size of 1cm treatment & given anti browning agents ascorbic acid citric acid 40mg dissolve the 100ml distilled water 45 minute. Per day noted the growth or contamination changes the media after 7 day all pant maintain but change the 14 day plant no growth all plant or no results.

Table no 2: Composition of MS media MSB1 and MSB2

Medium no	BAP Mg/l	IAA Mg/l	Kinetin Mg/l	pH	No of plant	Growth of plant	Death of plant	Day	Treatment
MSB_1 MSB_2	1.0 2.0	00	00	5.4	8	03	5	**10-14**	Bavistin 1gm/l corbendazim fungicide 1gm/l almox 10mg/l Citric acid and ascorbic acid 40mg/l

Table no 3 Laminar flow used of inoculation with suckers regular treatment. Sterilant use ethanol 70% 30 sec or 1 minute. The treatment of mercuric chloride 0.1 mg/100ml time 5 minutes, 6minutes and 8 minutes. Treatment of mercuric chloride 0.2% mg/100ml time 5 minutes, 6minutes and 8 minutes. Bavistin1gm/l corbendazim fungicide 1gm/l and almox 10mg/l five suckers successful.

Table no 3: Inoculation at Laminar Flow

S.No	Sterilant use	Concentration %	Time Minute
1.	Ethanol	70	30second, 1.0 minute
2.	Mercuric chloride	0.1mg/100ml 0.2 mg/100ml	5min,6min,8min 5min,6min,8min.
3.	Bavistin1gm/l corbendazim fungicide 1gm/l almox 10mg/l		Pretreatment after contamination or not treatment change the media

Pretreatment after contamination or not treatment change the media

Table no 4 shows composition 0f MS media MSB3 and MSB4 the effect of ascorbic acid concentration in present study, the extent of browning and death of explants were observed on weekly basis for four weeks. Generally, lethal browning increased with time, but decreased with increased concentration of ascorbic acid and citric acid 80mg/100ml then declined at highest concentration during this experiment, highest degree of lethal browning was observed in control treatment with sucker normally. In this experiment the use of ascorbic acid and citric acid during explants preparation showed the best results in controlling lethal browning throughout the experimental period. The analysis indicated that there were significant differences in the extent of lethal browning in control 28 day successful.

Table no 4: Composition of MS media MSB3 and MSB4

Medium no	BAP Mg/l	IAA Mg/l	Kinetin Mg/l	pH	No of plant	Growth of plant	Death of plant	Day	Treatment
MSB_3 MSB_4	3.0 4.0	00	00	5.8	10	00	4+4 7+8	**7+14** **21+28**	Ascorbic acid, citric acid 80mg/100ml

Table no 5 shows composition of MS media MSB5 showing effects of various concentrations of ascorbic acid citric acid and bavistincordendazimalmox in presents study ascorbic acid citric acid at concentrations of 100 mg/l showed good control.

The death of explants due to lethal browning was mostly observed in the control treatment. Generally the highest death of explants was observed on third and fourth successful treatments. The lowest survival of explants was observed in the control treatment where there were no explants which survived. The highest survival of explants was observed in treatment of soaking the explants for 45 minute 100 mg/100ml of ascorbic acid during explants preparation of the explants survived the incidence of lethal browning. This was followed by the use of ascorbic acid at concentration of100 mg/100ml applied directly to the media where of the explants survived and longtime no contamination. The use treatment of plant bavistin 1gm/l corbendazim 1mg/l almox10 mg/l 1hours treatment. Plant are regular no effect of contamination 10 day this treatment next MS medium.

Table no 5: Composition of MS media MSB5

Medium no	BAP Mg/l	IAA Mg/l	Kinetin Mg/l	pH	No of plant	Growth of plant	Death of plant	Day	Treatment
MSB_5	5.0	00	00	5.8	5	00	02 6x	10 12+15 15+25	Bavistin 1gm/l Corbendazim fungicide 1gm/l almox 10mg/l 100mg ascorbic acid citric acid

Table no 6 shows composition of MS media MSB6 BAP 5+6 mg/l IAA 0.1 mg/l Kinetin 0.1mg/l was used this plant raised was selected the healthy plant process of explants treatment shown Preparation and inoculation of explants (suckers) for mass multiplication plant variety Grande Naine Suckers of banana healthy. Excision of pretreatment of suckers with bavistin 1gm/l, Carbendazim 1gm/l and almox 10 mg/l for controlling of fungal and bacterial contamination.Selection of proper banana sucker's size for inoculation.Treatment the rhizome anti-browning agent citric acid and ascorbic acid 100mg for 45 minute.MS media preparation.Inoculation at suckers use in laminar flow. The suckers washed 2-3 time distilledwater. Treatment ethanol 70% 30 second was 2-3 time Distilled water. Treatment mercuric chloride 0.1mg dissolves 100ml 8 minute and washed 2-3 time distilled water inoculation with MSB6 media.

Table no 6: Composition of MS media MSB6

Medium no	BAP Mg/l	IAA Mg/l	Kinetin Mg/l	pH	No of plant	Growth of plant	Death of plant	Day	Treatment
MSB_6	5.0 6.0	0.1	0.1	5.8	5	5		1-28	Bavistin 1gm/l corbendazim fungicide 1gm/l almox 10mg/l 100mg ascorbic acid citric acid

Initiation of shoot

The sterile of media containing different concentration of BAP ranging from 1to 6 mg/l were studied. The initiation of shoot was observed after12 days of inoculation in each media. At subculture level the hardness of the tissue was observed in each shoots. Sprouting of shoot was low in media containing 1.0 mg/l BAP this hormonal concentration (Exide shoot deep with intact rhizome and leaf sheath explants of banana on MS medium growth of leaf sheath browning / blackening of rhizome. Growth of green leaf sheath unfolded after 7 days in MS medium.) Single leaf development of shoot was observed in media containing 2.0 mg/l BAP, same as observed in case of 1.0 mg/l BAP media. In media containing 3.0 and 4.0 mg/l BAP, sprouted shoot was observed relatively better developed conditions. At subculture level the hardness of the tissue was which rigidified material to use for manipulations in in vitro condition is observed change the media a growth of shoot in MS media containing 5mg/l BAP + 0.1 mg/l each of kinetin and IAA after 7 days). The initiation of 2-4 leaves with healthy development of shoot was observed in media containing 5.0 mg/l BAP Various in the length of shoot in the 5mg/l BAP +0.1 mg/l each of kinetin and IAA after 14 days, growth of shoot and white root primordial after 28 days of culture on rhizome surface in the medium containing 5mg/l BAP +0.1 mg/l each of kinetin and IAA), 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.

Sub culture for multiplication

After 12 days of Growth of the shoot 6 mg/l BAP + o.1 mg/l each of kinetin and IAA formation of shoot and root after 28 days culture) initiation, the shoots were cut at the base, separated and sub cultured to a fresh medium containing same hormonal concentration from where they are sub cultured. In major cases, after a week multiple shoots were developed from the sub cultured shoot. Multiple shoot formation was not observed in 1.0 and 2.0 mg/l BAP. Media containing 3.0 mg/l BAP while 4.0 mg/l BAP concentration shown 6-7 shoots of 4-5 cm height. The higher numbers of shoots were

observed in media containing 5.0 mg/l BAP concentration i.e. 8-10 shoots of 5-7 cm height. In media containing 6.0 mg/l BAP concentration, 6-7 shoots of 3-4 cm height were observed. Again subculture was done on a new fresh medium. Similarly in the present study also higher concentration of kinetin up to 5 mg/l showed healthy, long and good number of the shoots/explant. Therefore it is concluded that media containing 5 mg/l BAP showed optimum results with the variety studied. Shoot proliferation and multiple shoot development: For multiple shoot development, shoots were further cut with two gentle cross incisions upon apical meristem, separated and sub cultured in a fresh medium as described above. After a week of second subculture the number of auxiliary buds wasdeveloped. In 1.0 mg/l BAP containingmedia, slow growth and 3-5 shoots of 2-3 cm in length was recorded. In a medium containing 2.0 mg/l BAP also showed slow growth and 4-5 shoots of 3-5 cm in length formation of white colour root primordial rhizome surface in MS rooting medium after 21 and 28 days culture) per explant. In a medium containing 3.0 mg/l BAP showed 6-7 shoots of 4-5 cm in length and 4.0 mg/l BAP showed 5-6 shoots of 4-5 cm in length.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. Starting of shoot multiplication was observed after 7-8 days in final 5.0 mg/l BAP concentration. After 12 days of sub culturing, clumps of 5-7 shoots were observed with 4-5 cm height. The results were recorded after 5 days intervals, 8-10 shoots o Multiplication observed with 5-6 cm height, 10-12 shoots with 5-7 cm height. 12-14 with 4-7 cm height and 12-14 shoots with 6-8 cm height respectively after 15, 20, 25 and 30-40 days' time intervals. These shoots were suitable for root development.

Root induction

The auxins(IAA) are most frequently used to induce root initiation in the banana Rooting is also achieved on basal medium without any growth regulators. The influence of activated charcoal on rooting is when IAA was added to the medium in the presence of activated charcoal, no difference in rooting was observed. Rooting can be stimulated when individual shoots are transferred to basal medium alone.0.1 mg/l IAA Kinetin and activated charcoal (Geotropic formation of roots moving upwards with root hairs after 35 days of culture). After 10 days of transfer, 2.0 and 2.5 mg/l NAA containing medium had shown poor rooting. The use of 0.1mg/l and Kinetin rooting medium root development shown healthy growth of rooting with good quality numbers (Upward movement of roots with secondary roots hairs on the medium containing 5mg/l BAP, 0.1mg/l each of kinetin and IAA after 42 days of culture) 0.1mg/l IAA shows observation of healthy growth of rooting from elongated shoot after 15 days of transfer to rooting medium. While rooting and shoot elongation after 20 and 30 days. The plantlets after 30 days' time period were ready for hardening.

Hardening of tissue culture raised plants

Micro propagated plants were delicate in nature and sensitive to external environment as these plants were produced in closed sterile environment under controlled environmental condition and under high humidity and nutrient rich artificial synthetic media. These in vitro raised plants are incapable of fighting microbial attack including pathogens (Banana plant in pots after 30 days in nethouse). In vitro plants have to adjust external environment of reduced humidity, changing temperature and lower nutrient availability when shifted to free living environment. Not only have these plants limited photoautotrophic capacity so their energy requirement are met by reserves of starch accumulated during in vitro culture.

In vitro plants were removed from culture media under aseptic conditions. When rooted plants were removed from glass bottle (jars) some of the roots were very fragile and damaged while removing the medium from the roots surface in spite of due care. The whole plants were transferred to culture bottle having hormone free liquid media covered with cap for a period of 20 day in closed enclosures. Banana plant in pots after 30 days in net house. After 30 days of culture, a small hole was made on the top of culture bottle and such sterilized cap was plugged or cap was replaced having 10 mm hole fitted with cotton wool. This cotton plug was gradually removed and cultures were maintained for another week for hardening. It was observed that not a single plant was damaged or necroses by this method. These plants were transferred to soil in small size plastic pots.

CONCLUSION

The present study investigates following conclusions that are drawn from above results. The two cultivars shows differences in shoot commencement, shoot proliferation and rooting. Grade nine found to be more responsive and had highest rate shoot commencement, shoot proliferation of for in vitro techniques among the two cultivars. This may be due to geneticmakeup of two varieties.Alone BAPcannot effective for shoot commencement, shoot proliferation. In contrast to alone, combination of BAP and Kinetin wasfound most effective for shoot commencement, shoot proliferation. As far as rooting is concernedBAP showed best performance inboth cultivars of banana.In hardening the mixture of FYM+ Soil inratio (1:1) showed good survival rate inboth cultivars varieties of banana.Plantlets production through in-vitro planttissue culture techniques seems to bevery effective for rapid and large scaleproliferation of banana cultivars. The method is also effective for higher yield and had commercial benefits for banana production.

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