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Tissue culture studies in banana (*Musa paradisica* L.)

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ABSTRACT

Advance in cell tissue and protoplast culture in recent years has evoked great interest in geneticists and plant breeders to exploit techniques in gene manipulation and crop improvement. Crop improvement primarily depends on the availability of desired genetic variation. A general knowledge of media, specific organic requirements, source of explants and gene type that promotes efficient callusing and regeneration in long term cultures are prerequisites for successful exploitation of cell and tissue culture techniques for crop improvement: Of all the terms which have been applied to the process, "micro propagation" is the term which best conveys the message of the tissue culture technique most widely in use today(1). The prefix "micro" generally refers to the small size of the tissue taken for propagation, but could equally refer to the size of the plants which are produced as a result. Bananas are probably the only type of crop that is used a fruit and as a starch providing food. It is widely grown in most parts of the world. In many developing countries local people derive their food energy requirements from bananas. Improved banana programs can change banana production not only as a food but also as a cash crop for export, which would improve the living standard of the people The tissue culture involves producing disease free plants. This is a technique that enhances the ability of banana plant to regenerate into a whole plant from a single meristem or shoot apex.

Be multiplied into several thousand plants in less than one year. With most species, the taking of the original tissue explant does not destroy the parent plant.

The present work deals with cultivation of Musa Paradisica.

KEYWORDS

Micropropagation;
Meristem;
Explant;
Germplasm.

INTRODUCTION

Micro propagation is one of the important contribution of plant tissue culture to commercial plant propagation and has vast significance, for example -Banana tissue culture.. Micro propagation is the true to type propagation of selected genotype using in vitro culture technique[2]. This technique provides a rapid reliable system for a production of large number of genetically uniform disease free plantlets. The pioneering work in

this area of research was reported by Indian scientists. Today almost all the prime universities and institutes like BARC, Bombay, NCL, Pune, ISC Bangalore etc., in India are carrying out research in tissue culture.

The idea of cell and tissue culture was put forth by a German Scientist Haberlandt in 1902. All attempts to culture the plant cells and tissue were unsuccessful till the 1930 and around 1939 the possibility of culturing plant tissues for definite periods was independently reported by White, Nobecourt and Gautheret[5].

Hindustan Lever, Tata tea, Unicorn Biotech, Nath Seeds, RPG Enterprises, Indian Tobacco, Hindustan Agri Genetics Limited etc, are the major companies in the field or micro propagation in the country.

The technique of biotechnology like tissue culture has attracted the attention of farmers in the country.

In countries like India and Kenya, bananas are grown by small scale farmers with limited land for expansion. Through biotechnology, the little land can be made to

Chemicals and the hormones in the growth media make on meristem produce may tips which are then transferred to fresh media. After four weeks more meristems are produced. The cycle of multiplication can e repeated up to 10 times producing more than 1500 plants from a single initial meristem within a period of 40 weeks. It is vital that the initial meristem is free from any known diseases before multiplication commences, otherwise this could be multiplying the disease itself[3]. Choice of mother plant from the field is critical for the same reason. So, extending more than ten cycles is not desirable[6].

ADVANTAGES OF TISSUE CULTURE BANANA

- They are disease free and therefore grow faster and vigorously.
- They mature earlier, and the yield is more than conventional ones.
- Large numbers can be produced in a relatively short time occupying a relatively small space.
- Through banana tissue culture, development of new genotypes resistant to pests and diseases is possible. This can be extended to improved quality and storability.
- Banana tissue culture can facilitate safe international exchange of banana germ plasm from one country to another in a closed sterile environment free of diseases.
- There is a possibility of incorporating vaccines of common diseases in banana, as it is popular fruit throughout the world.

DISADVANTAGES OF TISSUE CULTURED BANANA

- The tissue cultured banana is expensive and so it is not reachable to the normal man.
- For the production of tissue cultured banana, it needs labor intensively.

PLANNING, DESIGN AND EQUIPMENTS

The setting up of a tissue culture laboratory needs proper planning. It depends upon space availability, volume of work to be carried out and funds. Usually, the availability of space, volume of work to be carried out and funds. Usually, the available laboratory space is been divided into five distinct laboratory areas.

- Media preparation area
- Aseptic transfer chamber area
- Environmentally controlled culture room
- Analytical room
- Acclimatization room

REQUIRED EQUIPMENTS

- All necessary glassware
- Magnetic stirrer
- Electronic balance
- Heating mantels
- Laminar air flow chamber
- Hot bath
- Auto clave
- Growth chamber
- pH meter
- Air conditioned room
- De ionizer and Distilled water unit.

MS- MURASHIGE & SKOOG MEDIUM- MEDIA STOCK PREPARATION

SOLUTION	COMPOSITION	VOLUME
A	NH_4NO_3	82.5 gm/lit
B	KNO_3	85 gm/lit
C	NA_2EDTA $FESO4, 7H_2O$	1.865 gm/lit 1.39 gm/lit
D	$CaCl.H_2O$	88 gm/lit
E	KH_2PO_4	34 gm/lit
F	H_3BO_3 $Na2MoO_4. 2H_2O$ $CoCl_2.6H_2O$ KI	1.24 gm/lit 0.05 gm/lit 0.005 gm/lit 0.166 gm/lit
G	$MnS0_4. H_2O$ $MgS0_4. 7H_2O$ $CuSO_4.5H_2O$ $ZnSO_4.7H_2 O$	4.46 gm/lit 74 gm/lit 0.005 gm/lit 1.725 gm/lit
H VITAMINS	Meso-inositol Thiamine-HCL Pyramidine Hcl Glycine Nicotonic Acid	0.lgm/lit 0.0lgm/lit 0.05 gm/lit 0.2 gm/lit 0.05 gm/lit

STOCKS	*FOR 1*	*FOR 10*
A	20 ml	200 ml
B	20 ml	200 ml
C	20 ml	200 ml
D	5 ml	50 ml
E	5 ml	50 ml
F	5 ml	50 ml
G	5 ml	50 ml
H	1ml	10 ml
CASINE	0.1 gm	1.0 gm
MESO INOSITOL	0.1 gm	1.0 gm
SUCROSE	30 gm	3000 gm
AGAR	8gm	800 gm
pH	5.8	
PVP	100 mg	10 gm

COMPOSITION OF MEDIA FOR BANANA

MACRONUTRIENTS	1 LITRE	5 LITRES
NH_4NO3	1650 mg	8250 mg
KNO_3	1900 mg	9500 mg
$MgSO_4, 7H_2O$	370 mg	1850 mg
KH_2PO_4	170 mg	850 mg
$CaCl_2$2H-0	440 mg	2200 mg

MICRONUTRIENTS I	1 LITRE	100 LITRES
H_3BO_3	6.2 mg	8250 mg
$CoCL_2.2H_2O$	0.025 mg	9500 mg
$CuSO_4.5H_20$	0.025 mg	1850 mg
$ZnS0_4.7H_2O$	8.6 mg	850 mg
$MnSO_4$	22.3 mg	2230 mg
Na_2MoO_4	0.25 mg	25 mg

Add 1 ml/lit

MICRONUTRIENTSII	1 LITRE	100 LITRES
KI	0.83 mg	83 mg

Add 1 ml/lit

Fe STOCK

Na_2EDTA	372.6 MG/50 ml
$FeSo_4$	278 mg/ 50 ml

Na_2EDTA ADD $FeSo_4$ Solution drop by drop

Add 1 ml/lit

VITAMIN STOCK

Nicotinic Acid	50mg/100ml
Pyridoxine. Hcl	50 mg/ 100ml
Thiamine. Hcl	100 mg/100 ml
Glycine	200 mg/100 ml

Add 1 ml/lit

FRESH WEIGHTS

Sucrose	30 gm/lit
Agar-Agar	8 gm/lit
Mesoinositol	100 mg/lit

Upto here it is called as BASAL MEDIUM

GROWTH HORMONES

BAP -Benzyl amino	3-4 Mg / lit dissolve in IN
IAA-Indole acetic acid	1 mg/lit dissolve in IN NaOH
PVP-Poly vinyl	100 mg/lit

Macronutrients - Freshly
MicronutrientsI 1 ml / lit
Micronutients II——- 1ml/lit
Fe stock————— 10 ml/lit
Vitamin Stock ————— 1 ml/lit

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GROWTH REGULATORS

1	TYPE	DISSOLVE IN
2	IAA-Indole acetic acid	Ethanol / IN NaOH
3	IBA-Indole butyric acid	Ethenol / IN NaOH
4	Kinetin	IN NaOH
5	NAA	IN NaOH
6	TDZ-Thidiazonium	Ethanol / DMSO
7	Zeatin	IN NaOH
8	2-lp -2-isopentenyl adenine	IN NaOH
9	GA_3 -Gibberlic acid	Ethanol
10	BA -benzyladenine	IN NaOH

FLOW CHART TO BE FOLLOWED FOR BANANA PLANT MEDIA

200 ml distilled water
+
Basic salts (Micro & Macro)
+
Fe EDTA (20 ml/lit)
+
Vitamins (1 ml/lit)
+
Glycine (1 ml/lit)
+
Multiple Hormone (1 ml/lit)
+
Meso-inositol (100 mg / lit)
+
Calcium D (20 mg/lit)
(Calcium panthonate)
+
Sucrose (30 mg / lit)
(test PH for 5.6)
500 ml distilled water (boil) (PH 7.0)

Agar (8 gm / lit)

↓

Make up to 1000 ml

↓

Pour in bottles(sterilized) about 50 ml/bottle

↓

Keep aside 2 days for testing

WHITE'S MEDIA

(For Invitro Rooting)

MACRONUTRIENTS	MG/LIT
MgS04. 7 H20	750
NaH2P04.H20	19
KN03	80
Micronutrients	Mg/lit
H_3BO_3	1.5
MnS04.4H20	5
$ZnS0_4.7H20$	3
$CuS0_4.5H20$	0.01
KI	0.75
Organic supplements	Mg / lit
(Thiamine Hcl)	0.01
(Pyramidine Hcl)	0.01
(Nicotonic acid)	0.05
Glycine	3
Sucrose	20 gm /lit
Charcoal	20 mg lit
Agar	8gm
P^H	5.8

WHITE'S MEDIA STOCK PREPARATION

SOLUTION	COMPOSITION	1 MG / LIT
A	$KN0_3$	80
B	NaH_2P0_4	19
C	H_3BO_3	1.5
D	$MgS0_4.7H_20$	750
	$MnS0_4.7H_20$	5
	$ZnS0_4.7H_20$	3
	$CuS0_4.5H_20$	0.01
E	KI	0.75
F	Thiamine Hcl	0.01
	Pyramidine Hcl	0.01
	Nicotic acid	0.05
	Glycine	3

SOLUTION	1 LIT
A	50
B	50
C	50
D	50
E	50
F	2

Different concentrations of BAP and IAA were used with the White's media for banana plant micropropagation.

GROWTH REGULATOR	CONCENTRATION MG / LIT
BAP	0.5
IAA	1.0
BAP	1.5
IAA	2.0

2.0 mg / lit was the suitable concentration for banana plant.

PREPARATION OF M.S MEDIUM

The macro nutrients are weighed correctly and dissolved in 200 m dis. H20 in a flask. Micronutrients are weighed and dissolved in 200 ml of d. H20 in another flask. The macronutrient solution and micronutrient solution are mixed together and stirred well.

Sucrose is then added to the solution and stirred well. The volume of the solution is made up to 950 ml by adding dis. H_20**[4]**.

PH of the medium is adjusted to 5.7 using 1 N NaOH or 0.1 NHCL. The final volume is made up to 1 lit by adding dis. H20.

The mouths of vessels are plugged with non-absorbent cotton and vessles are covered with cheese cloth. The vessels containing media is sterilized in a autoclave at 121° C for 40 minutes.

The medium is allowed to cool. Then vitamins, amino acid and harmones are added to it. The resulting medium is used for tissue culture.

If solid media is required the prepared media is distributed into flasks and 1 % agar is added to it. Then the media is sterilized in an autoclave. The sterilized medium is distributed to culture bottles and allowed to cool at room temperature. As a result, a semisolid nutrient medium is formed in the culture bottles.

PROCEDURE FOR ESTABLISHMENT OF BANANA BY PLANT TISSUE CULTURE

Selection of meristem from the mother plant

A healthy growing mother plant without any disease was selected and from this a meristem piece of length 2 inches was cut down with a sterile surgical blade.

This 2 inches piece of meristem was cut into two equal halves of 1 inch each with a sterile surgical blade and then each piece of 1 inch length was cut into four equal halves vertically and then this was cut into four divisions horizontally to get 16 pieces of meristem finally.

Surface sterilization

Shoot meristems collected from crops were surface sterilized by washing them under running tap water for 30 minutes followed by Bavitin for 20 minutes then rinsing with 1% Teepol for 10 minutes, followed by 10 minutes in 0.1 % Hg Cl2, then dip in 70% Ethnaol for 1 minute then go for inoculation.

Inoculation into establishment media

- The MS media with 6 mg/lit Benzyl amino purine (BAP) was used as the establishment media.
- This media was autoclaved and then about 20 ml of the medium was poured into each culture bottle.

Each piece of the meristem was inoculated into separate culture bottles with establishment media. This procedure was done in the year 2003.

Observations

Shoot multiplication was observed.

Callus was formed.

Inoculation into multiplication media :

The shoot multiplication in the above procedure was observed after 4 weeks of incubation,

Later these multiplied shoots are cut into equal parts and are incubated into 20 ml of multiplication media.

Observation

The shoot multiplications were observed further more.

Inoculation into elongation media

The shoot tips were further again inoculated into elongation media. This was used for further elongation of the shoot.

Observation

The shoot tips were seen elongated.

Inoculation into rooting media

The elongated shoot tips are further inoculated into rooting media (ie., the MS media + BAP 4 mg/lit +

IAA 6 mg/lit + 1% charcoal) and incubated for 1 month.

Observation

Rootings were observed on the shoots.

After the rootings were observed, the plantlets were'slowly removed from the medium and were washed under the running tap water.

And they were finally grown in a net pot in the Green House.

Hardening

Rooted, unrooted plants are transferred to a sterilized vermiculite and they should be gradually exposed to a lower humidity and a higher light intensity in mist chamber.

Rooting of cuttings and hardening of grafted seedlings is done.

RESULT

Micro propagation is one of the important contribution of plant tissue culture to commercial plant Propagation.

A healthy growing mother plant without any disease is selected. And the 2 inches piece of meristem surface sterilized and inoculated in MS media.

After inoculation the shoot multiplication is observed, callus was formed. The shoot multiplication is observed after 4 weeks of incubation . Later these multiplied shoots are cut into equal parts and are incubated into 20 ml of multiplication media. The shoot multiplication is observed further more.

The shoot tips were seen elongated. The elongated shoot tips are further incubated into rooting media and incubated for 1 month. Rootings were observed on the shoots and they were finally grown in a net pot in Green house. And they were exposed to a lower humidity and a higher light intensity in mist chamber[7].

CONCLUSION

Every country is taken as challenge to be part of global market with their tissue culture products. Billion dollars industry is based on the secondary products around globe. Now a day many countries are aimed to establish the tissue culture laboratories with all sophisticated equipment even in the nucon corners of the country.

In many developed countries and in most polluted areas are aimed on green house revolution which is ultimately aimed at tissue culture.

The scientist and nurserymen have to dedicate to develop the plants and their products, which really dosen't lead to wrong notions in common country side farmers. As banana is poor man's food, the demand for this is throughout the year.

Because "Farmers poverty is nations poverty and farmers welfare is nations welfare."

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