PROTOCOL FOR MICROPROPAGATION OF BANANA

Technical Report · January 2013

CITATIONS
0

READS
8,332

3 authors, including:

P.P. Joy
Kerala Agricultural University
151 PUBLICATIONS **421** CITATIONS
SEE PROFILE

Anjana R.
Kerala Agricultural University
13 PUBLICATIONS **10** CITATIONS
SEE PROFILE

PROTOCOL FOR MICROPROPAGATION OF BANANA

Joy P. P., Anjana R. & Prince Jose, Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686670, Muvattupuzha, Ernakulam, Kerala, India, Tel. & Fax: +914852260832, Email: prsvkm@gmail.com

Micro propagation of Banana

Plant tissue culture is a collection of experimental methods of growing large number of isolated cells or tissues under sterile and controlled conditions.

Banana is one of the world's most important fruit crop. It is grown in all type of tropical agriculture systems. The yield depends not only on the quality of soil and fertilization, but largely upon the control of the diseases.

An important objective of micro propagation of banana is to produce large number of disease free planting material and to satisfy the large need of banana planting materials.

Stages of micro propagation of banana

Selection of mother plant

⇩

Preparation of explants

⇩

Fresh inoculation

⇩

Multiplication

⇩

Rooting

⇩

Planting out

⇩

Primary hardening

⇩

Secondary hardening

⇩

Field planting

Diagrammatic Representation of Micropropagation of Banana

Selection of mother plant (0 day)

Banana Inflorescence

Preparation of explants

Fresh inoculation (0 day)

Multiplication (90 days)

Rooting (6 months)

Planting out & primary hardening (7 months)

Secondary hardening (8 months)

Field planting (9 months)

Joy P. P. 2013. Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686 670, Muvattupuzha, Ernakulam, Kerala
Tel.: 0485-2260832, 9446010905; Email: prsvkm@kau.in, prsvkm@gmail.com; Web: www.kau.edu/prsvkm, http://prsvkm.tripod.com

Selection of mother plant.

The micro propagation work can be facilitated by the strict selection of planting material at the onset of the production cycle. Suckers and inflorescences can be collected from field, gene banks, and farmer's field or from isolated nursery area. Criteria for the selection mother plant- they are disease free, high yield and good quality plants.

Preparation of explants and fresh inoculation

1. **Banana shoot tip (sucker)**

- Wash the suckers thoroughly in tap water, roots and leaf sheaths are removed, and basal portion of the corm is cut and trimmed to a size of12*12*15 mm.
- Keep the explants under running tap water for 30 minutes, then soaked in cleansole (detergent) for 30 minutes and are shaked continuously.
- Wash with distilled water to remove the detergent particles.
- Treat with fungicide (SAAF+INDOFIL) for 30 min followed by distilled water wash.
- They are then transferred to laminar air flow chamber for further sterilization process.
- Inside the laminar flow chamber, the explants are treated with 70% ethanol for 2 min,
- Wash with sterile water.
- After that the explants are treated with 0.1% Hgcl2 for 5 min
- Three rinsing of 5 minutes each with sterile water.
- The explants are trimmed to a final size of 8*8*10 mm, in sterile conditions inoculated on BA1 (MS+3mg/l BA1) media,
- Incubate at 25+/- 2 o C dark for 21 days.
- The media used for inoculation is changed after 21 days for 3 times, unless the phenolics released into the medium may inhibit the growth.

2. **Banana inflorescence**

- The bracts with the male flowers are removed until they become too small (3cm in length).
- Wash the inflorescences thoroughly in running tap water for 30 min
- Soaked in cleansole detergent to remove surface contaminants for 30 min in shaker.
- Wash with distilled water for about 5 times to remove soap solution.
- Fungicide treatment (0.05%Saaf+0.1 % indofil) for 30 min in a shaker.
- Wash with distilled water for 5 times to remove fungicide..
- Transfer to sterile bottles inside the laminar air flow chamber.
- Rinse with sterile water once.
- 70% ethanol wash for 2 minutes.
- Wash with sterile water once.
- Treat with 0.1%Hgcl2 for 5 minutes.
- Rinsing of 5 minutes wash with sterile water for 3 times.

OUTSIDE LAF

Banana sucker (explant)

Explant size minimized

Running tap water wash for 30'

Explants in soap solution for 30'

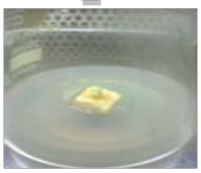
Treated with Bavistin(0.1%)+Indofil(0.2%)+SAAF(0.1%) for 30'

Washed out using distilled water

Further sterilization in LAF

INSIDE LAF

MULTIPLICATION



Inoculated to media

Cut the explants size to 8x8x10mm

Sterile water wash for 5'continuous shaking, thrice

0.1% $HgCl_2$ treatment for 5' with continuous shaking

Sterile water

70% alcohol wash for 2'

Transferred to sterile bottles

Fresh Inoculation of Banana shoot tip

Joy P. P. 2013. Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686 670, Muvattupuzha, Ernakulam, Kerala
Tel.: 0485-2260832, 9446010905; Email: prsvkm@kau.in, prsvkm@gmail.com; Web: www.kau.edu/prsvkm, http://prsvkm.tripod.com

OUTSIDE LAF

Banana inflorescence (Explant)

Explant size minimised

Running tap water wash for 30'

Explant in soap solution for 30'

Washed out using distilled water

Further sterilization in LAF

INSIDE LAF

MULTIPLICATION

Explant inoculated to media

Size minimized

Sterile water wash for 5' continuous shaking thrice

0.1% $HgCl_2$ treatment for 5' with continuous shaking

Sterile water wash

70% alcohols wash for 2'

Fresh inoculation of banana inflorescence

The inoculated explants will show bulging within 7 days, and may release phenolic compounds. Aftrer 21 days they are transferred to fresh media (BA1). About three media change will be done. After the third media change it will be subcultured to BA2 media for multiplication and formation of buds.

Multiplication

Multiplication step is for rapid production of clones. This step consists of separating shoots, culturing them up if they have grown bigger than optimum size, transferring shoot or sections of the shoot to fresh culture medium and again going through the same cycle of activities for another subculture. This step is repeated for seven to eight cycles.

Rooting

After the transfer of callus sections to BA2 media, the plants with 2-3 cm length are transferred to HB(half basal) media for the generation of roots.

Planting out and Hardening

Fully rooted plants in vitro are selected for planting out. Plants are first grown in mist chamber for acclimatizing with climate outside the lab. After 2-3 weeks time, they are moved to green house to get adjusted with field conditions. Plants are planted in potting mixture, after 5-6 days they are transferred to soil within a plastic cover. After 2-3 months the plants are ready for field planting.

For the planting of a mature hardened plant to the field, we require 9 months duration from the date of inoculation, and we get more than thousand plants from an explant that is inoculated in the artificial BA media.

Tentative production estimates (Nos)

Responding Explants	Period (Months) (If recovery ≥ 90%)			
	9	12	15	18
1	25	500	800	1200
5	125	2500	4000	6000
10	250	5000	8000	12000
50	1250	25000	40000	60000
100	2500	50000	80000	120000
500	12500	250000	400000	600000
1000	25000	500000	800000	1200000

One Biotechnologist & 6 racks for every 25000 TC plants (2500 bottles of 10 plants)
5750 TC plants (575 bottles of 10 plants) for every rack