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Micropropagation of Banana (*Musa acuminata* L.)

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SUMMARY

The present investigation entitled, 'Micropropagation of banana (Musa Acuminata L.) " was conducted at College of Agricultural Biotechnology,Saralgaon , during the academic year 2022-2023. The experiment was laid out in completely randomized are design with three replications for the availability of more number of planting material with highest degree of genetic stability in vitro conserve plants of Grand Naine varities of banana . In present investigation efforts were made to study the effective concentrations and time of exposure of effective concentrations of growth hormones auxins and cytokinin for in vitro regeneration of Grand Naine banana the results obtained during the period of investigation.

INTRODUCTION

Banana is a perennial herbaceous monocot which belongs to Musa genus of the *Musaceae* Family.and its botanical name is Musa *acuminata* L.It can be cultivated under subtropical conditions. Their origin is placed in southeast Asia , in the jungles of Malaysia, Indonesia or Philippines. In many developing countries of rural areas, banana plays significant roles as source of economic growth, income, food security and nutrition. Banana is one of the world's most important food crops. In India, banana crop accounts for 2.8 per cent of agricultural GDP. Bananas are one of the most consumed and cheapest fruits worldwide: they are the most traded fruit and the fifth most traded agricultural product. The global export value of the banana trade was estimated to be US $8 billion in 2016, with a retail value between $20 and 25 billion **.**Banana varieties that are hybrids with AAB and ABB genome constitutions are a staple food for a billion people in Asia and Africa and have 2n=3x=33 chromosomes. Edible bananas have 22, 33 or 44 chromosomes, representing diploid, triploid and tetraploid cultivars (Stover and Simmonds 1987. Most banana cultivars are triploid seedless parthenocarpy clones derived from hybridization between Musa acuminate. Genome size of banana is 523 Mbp. Optimum temperature is 27ºC. Soils with good fertility and assured supply of moisture are best suited. One medium ripe banana, provides about 110 calories, 0-gram fat, 1 gram protein, 28 grams carbohydrate, 15 grams sugar (naturally occurring), 3 grams fiber, and 450 mg potassium.The application of various biotechnological approaches has become an integral part of the banana industry now days (Vuylsteke, 1998). Hence, in vitro culture technology is proved as best alternative for the production of large numbers of planting material of banana in shorter time, lesser space, disease-free plants and adequate germplasm preservation. The present study was done by using following objectives by use of grand nine variety of Banana. 1.To study the effect of growth regulator BAP and IBA on propagation of Banana (*Musa acuminata* L.) 2.Optimization and sterilization protocol for in vitro propagation of Banana (*Musa acuminata* L.) 3.To standardized of protocol micropropagation of Banana (*Musa acuminata* L.)

Material and Methods:

1.Explant Source : The required Explant (Sucker) of banana

2.Explant Preparation: The plant material of banana C.v. Grand naine (Musa acuminata) were used collected from college farm .

3.Chemicals: The details of the various laboratory and biochemical components used in the present investigation for media preparation and surface sterilization of the explants are given below:

A.Chemicals For Media Preparation: Agar**,** Myo-inositol ,3.1N HCl**,**1N KOH

B.Chemicals For Surface Sterilization: -Bavistin,70% Ethanol, Mercuric chloride , Tween20

C.Plant Growth Regulators: -1.BAP 2.IBA

D.Glassware's: Beaker**,** Culture Bottles, Test Tubes, Conical Flask, Glass Funnel, Glass pipette**,** Glass Rod**,** Measuring Cylinder

4.Equipment's : Autoclave**,** Hot plate, Laminar air flow**,** Magnetic Stirrer**,** pH meter**,** Weighing balance

5.Other's: Aluminum Foil, Bag, Cotton, Filter Paper Disc, Micropipette**,** Spatula**,** Spirit**,** Syringe**,**Tips**.**

Experimental conditions –

All in vitro studies were carried out ascetically in laminar airflow chamber the experiments were conducted under well-defined conditions of culture room maintained at 25°C temperature uniform light 1600 Lux provided by Fluorescent tubes over a night and dark cycle of 16/8 hours.

Aseptic techniques –

The standard sterilization techniques was followed as suggested by street 1997 For inoculation and sub culturing of explants in culture bottles. Inoculations of was carried out under a septic conditions in laminar airflow bench during the course of transfer of explants all surgical instruments were dipped in alcohol and gas chamber and explants cooled Before.

Preparation of explants for culturing –

The plant material obtained from the field was thoroughly washed under running tap water next plants were trimmed away using stainless steel knife until the length of explants is about 4 to 6cm and diameter at leaf base is 2 to 4cm is Shoot tips were collected in a tray and kept in water for five minutes then explants where pretreated with Dettol, savlon, tween 20 as per following table then explants we are placed in running tap water for one hour transfer the explants in laminar airflow and various pretreatment step.

Inoculation of Ex plants for establishment shoot initiation and shoot multiplication –

The usual procedure of Dissection and disinfection as described by cronauer & krikorion 1984 was used the suckers were trimmed further to the final size of 2-3 cm & inoculated in bottles MS medium supplements with various growth hormones using aseptic culture techniques the cultures were incubated in a room initially the cultures were kept up to 21 days on the establishment medium for initial 6-14 days cultures were incubated in dark & then transferred into light 1600 lux conditions for 15 days All in vitro studies were carried out aseptically in laminar air flow chamber. The experiments were conducted under well-defined conditions of culture room maintained at 25 ± 20C temperature, uniform light (1600 Lux) provided by fluorescent tubes (7200 K) over a light and dark cycle of 16/8 hours.

Media Preparation:

The culture medium used in the present study was prepared with references Murashighe and Skoog [MS, 1962] media. Sucrose was added to MS medium with growth regulators to promote multiple shooting. After adding growth regulators pH of the medium was adjusted to 5.8±0.1, the agar agar was added at concentration 8 gm/lit. The media was autoclaved at 121˚C and 15 psi pressure for 20 min.

Methodology:

Collection of explant (sword suckers)**.**Wash under running tap water Explant trimmed using stainless steel knife (about 2 to 5 cm)**.**Then explant placed in running tap water for 1 hr.**,.** Pre-treatment: Tween-20 (0.5%) for 10 min & Savlon (1%) for 10 min then wash with D/W- 2 times**.** After pre-treatment, bring explants in laminar air flow and treat with following sterilizing agents**.** Carbendazim and wash with distilled water for 2 times**.**70% ethanol (1min) treatment. Treat explants with various sterilizing agents for different durations**.** Wash with double distilled water 3 times**.** Inoculation of explants in MS medium**.** Incubation condition (16 hr photo-periodic time).

Result:

The maximum number of shoot where established in media combination containing MS4.5 mg per L BAP 0.170 5mg per L IAA20 mg per Ascorbic acid. The media combination MS4 .5 mg per L BAP 0.170 5mg per L a 20 mg per L ascorbic acid was found effective for Maximum number of shoot initiation and a maximum rate of multiplication shooting. Explants used throughout this experiment were excised from field grows suckers word suckers) of red banana. The plant material obtained from the field was thoroughly washed under running tap water. The explants were d by using stainless steel knife until the length of explants is about 4 to 6 and diameter at leaf base is 2 to 4 cm. These shoot tips were collected in a tray and kept in water for 5 min, then explants were pretreated with dettalrin and Tween 20 with different concentration and various time interval as per .Further

explants were placed in running tap water for 1 hr. These plants were transferred in laminar air flow and various pre-treatment steps are followed and observations were recorded .

Sr. No.	Media combination	No. of explants inoculated	Percent response	Days require for induction
1	MS basal	10	12.133 (20.37)	55
2	MS + 1 mg/L BAP	10	23.5 (28.98)	52
3	MS + 2 mg/L BAP	10	34.667 (36.05)	49
4	MS + 3 mg/L BAP	10	54.033 (47.29)	48
5	MS + 4 mg/L BAP	10	59.067 (50.20)	42
6	MS + 5 mg/L BAP	10	89.533 (71.11)	39
7	MS + 6 mg/L BAP	10	74.033 (59.34)	43
		C.D.	2.479 (1.59)	
		S.E.	0.809 (0.52)	

Table No.1: Effect of different concentrations of growth regulators on culture of Grand naine Banana .

Fig. 1. Inoculation

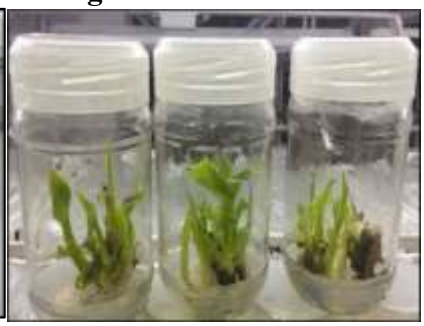

Fig. Multiplication

CONCLUSION

The media combination MS4 .5 mg per L BAP 0.170 5mg per L a 20 mg per L ascorbic acid was found effective for Maximum number of shoot initiation and a maximum rate of multiplication shooting.

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