Bioscience Discovery, 11(2):90-94, April - 2020

Open Access, Peer Reviewed, Referred Research Journal

Print & Online available on https://jbsd.in
ISSN: 2229-3469 (Print); ISSN: 2231-024X (Online)

Research Article

Studies on Recycling of Used Agar for *In Vitro* Propagation of Banana CV. Grand Naine

Sherkar Sandip Harishchandra, Anita Sharma, Sherkar H.D. and Pankaj Rathod

Genuine Biosciences, Gut No 121/2, Sambhaginagar, Dist-Osmanabad MH-43 501.

Article Info

Received: 20-12-2019,
Revised: 26-02-2020,
Accepted: 27-02-2020

Keywords: Agar-agar, used agar media, recycling, re-melting.

Abstract

The nutrient media (Murashige and Skoog 1962) is widely used in micropropagation of banana cv. Grand naine and discarded after the use. A simple, efficient, economical and highly reproducible protocol is developed for the *in vitro* propagation of banana. In the present study we collected used agar, recycled and used to prepare MS media. It was found that the control media and MS media supplemented with 4 mgs BAP and 10 mgs $AdSO_4$ along with used agar media showed good shoot multiplication (1:2.51 and 1:2.34) and shoot height. All the three media MS + IBA (control), MS + used agar + IBA, and used agar alone + IBA showed 100 percent rooting response. The average length of roots is almost similar (40.567 mm, 39.567 mm, and 38.333 mm).

INTRODUCTION

All the universities, research institutes and commercial laboratories are carrying out their research and production using microbial and plant nutrient media containing various essential elements. Murashige and Skoog (1962) medium are being used for the multiplication of horticultural, floricultural, forestry and medicinal plants in plant tissue culture laboratories. In order to increase awareness among researchers and scientists, innovative techniques are needed to lower down the cost of tissue culture plants. Low cost option at various growth stages of *in vitro* plant propagation results in lowering the cost of production without affecting the quality and quantity of plants (Prakash *et al.*, 2004). Banana plant production using low cost options is achieved by improving existing processes and utilization of available resources is reported by Savingikar (2002).

Agar is a high molecular weight polysaccharide extracted from various species of red algae (Rhodophyaceae) including *Gelidium* and *Gracilaria*. Generally, it is used as gelling agent for the solidification of nutrient media because it does not react with any components of medium and is not digested by enzymes from plant tissue. Chemically it is a polymer with subunits of galactose (Ahmed and Khan, 2014). It dissolves in boiling water at 80°C and becomes semi-solid at room temperature. It is resistant to microbial digestion and liquefaction and hence used not only for laboratory purposes but also for thickening purposes at food industries. In plant tissue culture industries for gelling of culture media it is used at concentration of 0.6 - 0.8%. Most of the commercial research laboratories are searching for substitute for gelling agent agar, as it costs about 40% to 50% of media. Once solidified nutrient medium is used, then it is discarded which serves as growth supplements to deleterious microorganisms. At each laboratory, huge amount of used agar media is discarded without treatment. Used agar is resistant to microbial digestion which remains in the environment as such for long time without disintegration and hinders the other forms of life (Armisen and Galatas, 1987). The recycling of used agar media and then reusing it in the laboratory or for converting it to biofertilizer is of great importance. As banana is the fourth most important fruit crop, most of the plant tissue culture laboratories are engaged in the production of disease-free planting material which generates

large quantity of agar waste. Hence recycling and reusing of used agar media will be cost effective as well as helps in saving environment. The aim of study is to develop protocol for recycling and reuse of used agar media for *in vitro* propagation of banana cv. Grand Naine.

MATERIALS AND METHOD

Plant material: In the present studies sterile cultures of banana cv. Grand Naine maintained at Genuine Biosciences research laboratory, Osmanabad, Maharashtra, were used as a source of explants material throughout experiment.

Collection, washing and remelting of used agar: The used agar was collected from the 4 weeks old sterile banana multiplied cultures after use. This used agar was removed from bottles and collected in container on the same day of subculture.

The used agar was then washed off using RO water so as to remove phenolics and plant pieces remained on used media pieces.

The washed used agar was then poured into clean conical flask which was then transferred to pressure cooker for remelting. The used agar was heated for 20 minutes in pressure cooker under high pressure. The remelted used media was again filtered using muslin cloth.

Media preparation: The stock solutions required for Murashige and Skoog (1962), vitamins, hormones (6-benzyl amino purine and Indole 3-buryric acid) and $AdSO_4$ was prepared well in advance and stored in refrigerator. Total six different types of media were prepared.

Multiplication media: 1) MS basal medium supplemented with 6 g agar and 30 g sucrose, used as a control, 2) 500 ml of full strength MS basal medium supplemented with 3 g agar and 30 g sucrose + 500 ml used agar medium, 3) Used agar medium fortified with 30 g sucrose only. All above three media were supplemented with cytokinin-BAP (4 mg) and $AdSO_4$ (10 mg). These media were used for *in vitro* multiplication of banana cv. Grand naine.

Rooting media: 1) MS basal medium having 6 g agar and 30 g sucrose, used as a control, 2) 500 ml of full strength MS basal along with 3 g agar + 500 ml used agar medium having 30 g sucrose, 3) Used agar medium fortified with 30 g sucrose only. All above three media were supplemented with of auxin-IBA (1 mg) and activated charcoal (100 mg). These media were used for *in vitro* rooting of banana cv. Grand naine.

The pH of all these six media was adjusted to 5.8 prior to addition of agar using 0.1N NaOH or 0.1N HCl. All the media were boiled to dissolve all media components by constant stirring. This medium was then dispensed (40 ml/ bottle) in culture bottles. These media bottles were sterilized in an autoclave at 121ºC and 1.02 kg pressure for 20 minutes. The data was recorded for media colour, initial pH of prepared medium prior to pH adjustment, media strength and contamination of media after sterilization (Table-1).

Inoculation of explants: Three clumps per bottle, each with two shoots was dissected and inoculated vertically on different multiplication media. The data was recorded for multiplication rate, average height of shoots, quality of shoot, and average fresh weight of clump (Table-2). Eight single shoots per bottle were inoculated on different rooting media for *in vitro* root induction. The data was recorded for per cent rooting, average length of roots, average number of roots/shoot and average height of shoots (Table-3).

Cultural conditions: Each culture bottles were labeled and sealed using wrapping film to avoid contamination and incubated in plant growth room**.** The temperature maintained was 28 ± 2ºC and the photoperiod maintained was 8 hours light and 16 hours dark throughout experiment. The light was provided using 20 wt. LED tube lights.

Experimental design: Data collected in the Completely Randomized Design (CRD) of experiments (Gomez and gomez) was analyzed using OPSTAT (14.139.232.166/opstat/), CCS HAU, Hisar. Each treatment consists of a set of 10 cultures which was replicated thrice; therefore, data represent's degree of variation in terms of Critical Difference (CD) and Standard Error (SE) at 5% values of 30 cultures. Data was subjected to one way Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

Appearance of prepared media: In the entire nutrient media, control as well as media prepared utilizing used agar media showed good gelling strength but the colour of media prepared was changed (Table-1). The re-melted used agar medium showed somewhat browning compared to control media due to repeated heating but solidifying strength is same. All the media showed different pH prior to pH adjustment (4.1 to 4.9). There was no microbial contamination observed in all the media before inoculation of explants.

Table 1: Influence of re-melting and sterilization of used agar media.

Sr. no.	Medium type	Initial pH (prior to pH adjustment)	Colour	Media strength	Per cent contamination after sterilization
1	MS + BAP (control)	4.9	White and translucent	+++	00
2	MS + Used Agar + BAP	4.7	White and translucent	+++	00
3	Used Agar + BAP	4.2	Light brown and semi-translucent	+++	00
4	MS + IBA (control)	4.5	White and translucent	+++	00
5	MS + Used Agar + IBA	4.1	White and translucent	+++	00
6	Used Agar + IBA	4.3	Light brown and semi-translucent	+++	00

* +++: Good gelling; ++: Moderate gelling; +: Poor gelling; -: No Gelling

Culture multiplication and growth: The rate of shoot multiplication was good (2.51) in control media supplemented with 4 mgs BAP and 10 mgs $AdSO_4$, similar results was reported by Muhammad et al. (2007) who observed best response of shoot multiplication in 4 mgs/lit BAP and 2 mgs/lit IAA while Rahman et.al. (2005) achieved highest multiplication on media supplemented with 4 mgs/lit BAP and 2 mgs/lit IAA and NAA, respectively.

However, in our experiments higher level of BAP 4 mgs/lit and 10 mgs $AdSO_4$ incorporated in used agar media was found to most suitable for banana cv. grand naine multiplication showing good multiplication rate (1:2.34 and 1: 1.89) respectively (Table-2, Fig-1).

Table 2: Influence of used agar on *in vitro* multiplication of banana cv. Grand naine (Data were recorded after 4 weeks of inoculation)

Sr. no.	Media type	Multiplication rate	Average height of shoots (mm)	Average fresh weight of clump (g)	Quality of shoot
1	MS + BAP (control)	2.51	32.50	3.16	+++
2	MS + Used Agar + BAP	2.34	31.33	2.9	++
3	Used Agar + BAP	1.89	31.50	1.93	+
	$CD_{0.05}$	0.21	0.80	0.28	
	SE	0.06	0.22	0.07	

* +++: good quality, ++: moderate quality: +: not satisfactory. CD: Critical difference; SE: Standard error

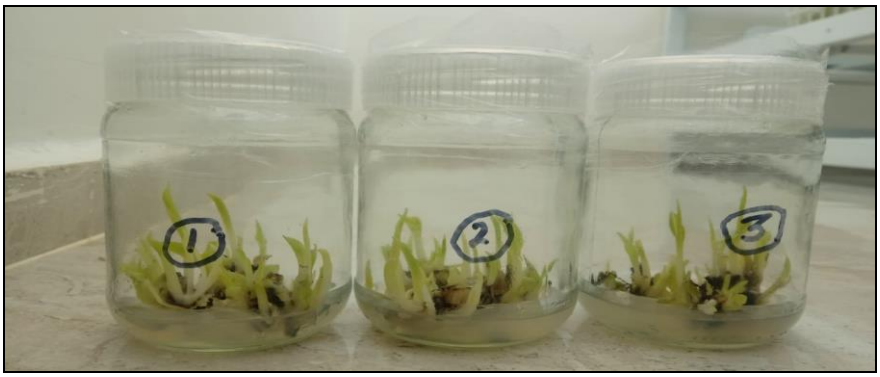

Figure- 1: Multiplication of banana cv. Grand naine on different media 1) MS + BAP (control), 2) MS + Used Agar + BAP, 3) Used Agar + BAP.

***In vitro* rooting:** In our experiment it is observed that in all media we achieved 100% rooting response (Table-3, Fig- 2). The same results were achieved by Gubbuk et.al. (2006) and Roy et al. (2010) who reported *in vitro* rooting on media supplemented only with 1.0 mg/lit IBA and 200 mgs/ lit activated charcoal. The addition of activated charcoal in banana rooting media increased rooting percent, enhanced time taken for root induction and shoot growth with well developed shiny leaves.

Table 3: Influence of used agar on *in vitro* rooting of banana cv. Grand naine (Data were recorded after 4 weeks of inoculation)

Sr. no.	Media type	Per cent Rooting	Average length of roots in mm	Average number of roots/shoot	Average height of shoots in mm
1	MS + IBA (control)	100 %	40.567	3.73	54.33
2	MS + Used Agar + IBA	100 %	39.567	3.86	53.63
3	Used Agar + IBA	100 %	38.333	3.46	51.83
	$CD_{0.05}$		1.325	0.23	0.97
	SE		0.376	0.06	0.27

CD: Critical difference; SE: Standard error

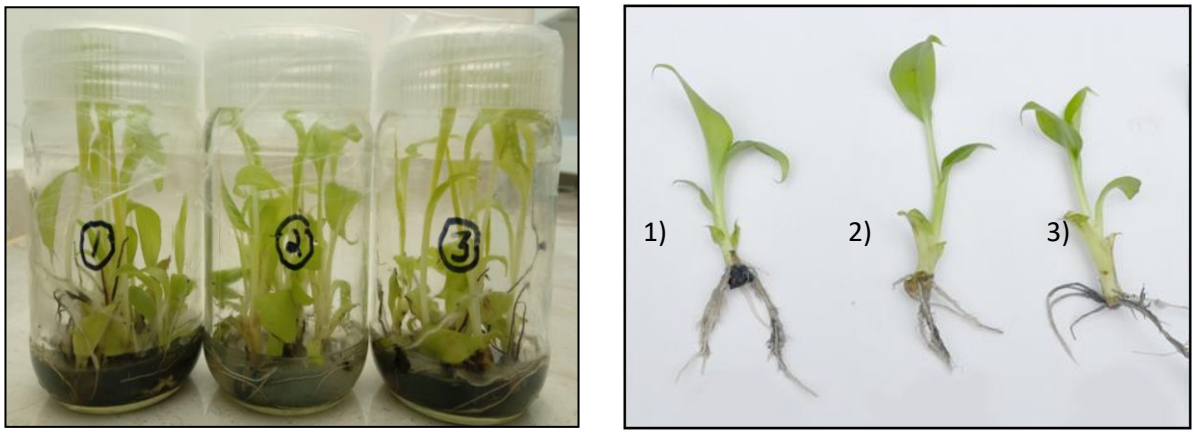

Figure- 2: *In vitro* rooting of banana cv. Grand naine on different media 1) MS + IBA (control), 2) MS + Used Agar + IBA, 3) Used Agar + IBA.

Conclusion: Agar, which is costly component of nutrient media, is easily reusable. Remelting of used agar showed change in color but no change in gelling strength of agar. It is concluded that the nutrition of culture media is not completely utilized during previous growth cycle. The reuse of used agar media showed no harmful effects on both *in vitro* multiplication and rooting of banana cv. Grand naine. The multiplication rate of culture was poor in media prepared by using used agar only compared to control media. Whereas, all rooting media were found to be equally effective in inducing roots. It is concluded that the used agar media can be recycled and reused for micropropagation of banana cv. Grand naine. The cost of banana rooting media can be reduced upto 40 to 50 % by utilizing used agar media. The used agar media needs to be analyzed for purity and porosity, since it may be decreased due to remelting (Meinita *et al.*, 2017).

REFERENCES

Ahmed SZ and Khan M, 2014. Recycling of Culture Media by Re-melt & Re-plating Method. *International Journal of Scientific and Engineering Research,* **5** (1):2159-2162.

Armisen R and Galatas F, 1987. Production, properties and uses of agar. *Production and utilization of products from commercial seaweeds. FAO Fish. Tech. Pap*, *288*, 1-57.

Gomez KA and Gomez AA, 1984. Statistical procedures for agricultural research. John wiley and Sons, New York. pp. 357- 427.

Gübbük H and Pekmezci M, 2004. *In vitro* propagation of some new banana types (Musa spp.). *Turkish Journal of Agriculture and Forestry*, **28**(5):355-361.

Meinita MDN, Marhaeni B, Hong YK and Jeong GT, 2017. Enzymatic saccharification of agar waste from *Gracilaria verrucosa* and *Gelidium latifolium* for bioethanol production. *Journal of applied phycology*, **29**(6):3201-3209.

Muhammad A, Rashid H, Hussain I and Naqvi SS, 2007. Proliferation-rate effects of BAP and kinetin on banana (Musa spp. AAA Group) 'Basrai'. *HortScience*, **42**(5):1253-1255.

Murashige T and Skoog F, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum*, **15**(3): 473-497.

Prakash S, Hoque MI and Brinks T, 2004. Culture media and containers. *Low cost options for tissue culture technology in developing countries*, p.29.

Rahman MZ, Rahman MH, Mullah MU, Nahar N, Sultana RS, Bari MA and Hossain M, 2005. Shoot Multiplication and Rooting of a Dessert Banana (Musa sp cv. Anupom). *Sciences*, 8(9):1298-1302.

Roy OS, Bantawa P, Ghosh SK, da Silva JAT, Ghosh PD and Mondal TK, 2010. Micropropagation and field performance of 'Malbhog' (*Musa paradisiaca*, AAB group): a popular banana cultivar with high keeping quality of north east India. *Tree and Forestry Science and Biotechnology*, **4**(1):52-58.

Savangikar VA, 2004. Role of low cost options in tissue culture. In *Low cost options for tissue culture technology in developing countries. Proceedings of a Technical Meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture*.11-15.

How to cite this article

Sherkar Sandip Harishchandra, Anita Sharma, Sherkar H.D. and Pankaj Rathod, 2020. Studies on Recycling of Used Agar for *In Vitro* Propagation of Banana CV. Grand Naine. *Bioscience Discovery***, 11**(2):90-94.

Google Scholar citation: https://scholar.google.co.in/citations?user=vPzEyC8AAAAJ&hl=en