INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

# EFFECT OF GELLING AGENTS IN *IN VITRO* MULTIPLICATION OF BANANA VAR. POOVAN

[a]Ramesh Y & [b]Ramassamy V
[a]Tissue Culture Laboratory, Perunthalaivar Kamaraj Krishi Vigyan Kendra, Puducherry – 605 009, India
[b]Post Graduate Department of Botany, Mahatama Gandhi Govt.Arts College, Mahe, UT Puducherry, India
Corresponding author email:rameshyouvarajan@yahoo.com

**ABSTRACT**
This study was aimed to examine the effects of different kinds of gelling agents in the tissue culture of banana var.Poovan. Three types of gelling agents namely Agar agar (Himedia), CleriGel (Himedia) and Gelwell (Microxpress) were used as supporting matrices and the performance of the banana cultures was assessed. There were significant differences in terms of shoot length, number of leaves and number of shoots produced per culture among the supporting media used. Best results were obtained with Gelwell (Microxpress) with 4.01 shoots per culture, while Agar agar and CleriGel containing media resulted in 2.2 and 2.53 shoots per culture respectively. The shoot length (3.96 cm) was the highest in Gelwell (Microxpress) followed by CleriGel 3.01 cm and Agar agar (2.84 cm).

**KEY WORDS**: *Gelling agents, Agar agar, CleriGel, Gelwell, MS 230, BAP*.

## INTRODUCTION

Banana belonging to the family Musaceae is an important and staple fruit crop grown widely in many countries. Because of its multifaceted uses and high economic returns it is referred to as "Kalpatharu" (a plant of virtues) (Singh HP, 2008). It is the most important fruit crop of India in terms of Production and Utilization. Conventionally, suckers are used as a propagating material in banana cultivation which usually houses pests and pathogens. There was a great challenge to over come the diseased planting material which often spread through vegetative propagation. Much efforts were put to produce disease free planting materials on a large scale through tissue culture which has quicker advantages such as high multiplication rates and high quality planting materials. Many studies have been reported on the micropropagation of *Musa* spp. (Cronauer and Krikorian, 1984; Wong, 1986; Nandwani *et al.*, 2000; Kodym and Zapata-Arias, 2004; Mohammad *et al.*, 2004.) which has resulted in the technological development and commercial production of many banana cultivars.

## MATERIALS & METHODS

### Selection of Explant

The banana sword sucker cultivar Poovan which served as explant source was collected from a local commercial banana grower in Puducherry region.

### Explant preparation and disinfection

The sword suckers were carefully removed from the mother plant without any damage and washed thoroughly in running tap water. Older leaves and the extraneous rhizome tissue were carefully chopped with a stainless steel knife. Trimmed suckers were then soaked in a solution of Bavistin (0.5%) for 1 hour. Shoot tips containing several sheathing leaf bases enclosing the axillary buds with subjacent rhizome tissue and measuring 4-5 cm in length were isolated. These shoot tips were soaked in a solution of Cetrimide (0.1%) – a bactericidal surfactant, and surface-sterilized with distilled water for 5 minutes. Further operations were carried out under a laminar air flow chamber. Cut surfaces of the rhizomatous tissue and leaf bases were further removed after the treatment of Mercuric chloride solution (0.1%) for 5 minutes by repeatedly washing several times with sterile distilled water. The explants measuring 2 cm were inoculated in the test tube containing the liquid medium. After 2 weeks, the healthy, contamination-free explants were swollen and turned green showing morphogenetic activity. They were removed from the liquid medium and the overlapping leaf sheaths along with the discolored tissue were removed aseptically. They were then splitted into two equal halves longitudinally and transferred to semi-solid medium substituted with Agar agar as a gelling agent (0.8%). Within 4-5 weeks time, the splitted explants began to proliferate with tiny protuberances heralding the progenitors of multiple shoots. These shoots were repeatedly sub-cultured on MS-230 semi-solid medium at an interval of 4 weeks per sub-culture. Uniform shoots were collected from the sub-cultured banana cultivar Poovan for this study (Figure 1).


Mother Culture


Explant


Uniform shoots

**FIGURE 1:** Selection of uniform banana shoot explants

**Media Preparation**

MS basal medium (Murashige & Skoog, 1962) was used for culturing banana Growth regulators- Benzylaminopurine (BAP) – 2 mg/litre and Adenine sulphate ($Ad.So_4$) – 30 mg/litre were added along with sucrose 3%. pH was adjusted to 5.8 before boiling. Gelling agents such as 8 gm/litre Agar agar (T1) and 2 gm/litre Gelwell (T2) and CleriGel (T3) was added separately to the media and allowed to boil for solubilization. The boiled media were poured in equal quantities to test tubes @ 15 ml / test tube and tightly closed with test tube caps. The media containing test tubes (T1-T3) were then autoclaved at 121$^{o}$C for 20 minutes.A longitudinal incision was made keeping the corm base intact and piercing the meristem of the culture to break the apical dominance. Then the uniform shoot bits were individually inoculated in the test tubes containing treatment media (T1-T3) by keeping the corm base completely immersed in the medium (Fig.2). These whole processes were carried out inside the laminar air flow chamber under aseptic conditions.

A total of 10 cultures per treatment were inoculated and arranged them in the sterile stainless steel test tube stands and incubated in the incubation room with a photoperiod of 16/8 hours light/dark and a light intensity of 3000 lux at a room temperature of 26$^{+/-}$ 2$^{o}$C. Average of 10 cultures / treatment was taken for shoot length, number of shoots and number of leaves at the end of fourth week.

**RESULTS & DISCUSSION**

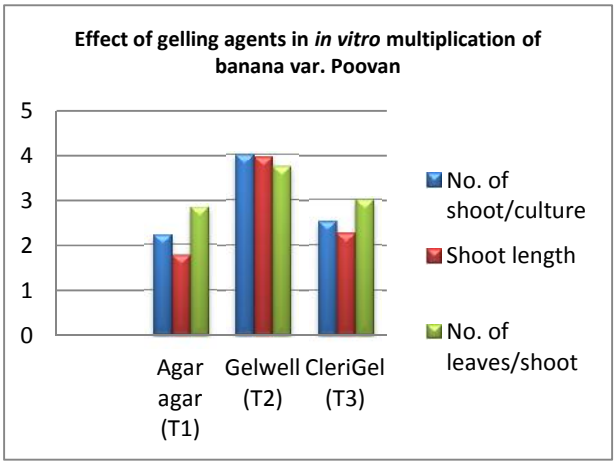
The effects of three commercial grades of gelling agents (Agar agar, CleriGel, Gelwell) were compared in the present study. Their effects on *in vitro* multiplication of banana var.Poovan had a significant changes on the parameters studied such as number of shoots, number of leaves and shoot length per plantlet (Figure 2).

**Number of shoots:** 100% shoot development was observed in all the three treatments carried out. But the number of shoots per culture was affected by gelling agent added. Gelwell increased the number of shoots (4.01/culture) followed by CleriGel (2.53/culture) and Agar agar (2.22/culture) (Table 1)

**Shoot length:** The cultures derived from Gelwell showed enhanced shoot length (3.96 cm) than the shoots derived from CleriGel (2.27 cm) and Agar agar (1.78 cm) (Table 1) (Fig.2).

**Number of leaves:** Number of leaves per shoot varied significantly in different gelling agents used in the present study. It was greater in Gelwell (3.75) followed by CleriGel (3.01) and least in Agar agar (2.84) (Table 1) (Fig.2).

It was found from the present study that Gelwell (Microxpress) which was stated equalent to Phytagel has beneficial effect on shoot multiplication and growth in the micropropagation of banana var. Poovan. In the earlier study, Kaçar *et al.*, (2010) reported that phytagel had higher number of shoots than agar in *in vitro* multiplication of Dwarf Cavendish bananas. In a similar study, Ramesh *et al.*, (2014) compared the effect of five commercial grade gelling agents on *in vitro* multiplication of banana var. Robusta reporting that gel medium (CleriGel) with table sugar as carbon source performed well than Agar agar with highest number of shoots and greater shoot length. Similar observations have been made in the beneficial effects of phytagel on shoot proliferation and growth in *Rosa damascena* Mill. and *Rhynchostylis retusa* (L.) by Kumar *et al*., 2002. Even though, agar is most frequently used as gelling agent in plant tissue culture, it has been reported to differ from batch to batch (Debergh,1983) and subsequently show variation in responses due to interaction with media components (Romberger *et al*., 1971), impurities (Nairn *et al*, 1995) and gelling strength (Debergh, 1983).




The accelerated shoot growth in the gel medium may be due more availability of water in the media than in agar. The most prominent distinction among the gelling agents which influences the *in vitro* growth characters is the water retention capacity of the gels and the availability of nutrients to the cultured tissue. Bornman and Vogelman (1984), Singha *et al*., (1985) and Ghashigaie *et al*., (1991) also reported that the absorption of cytokinin and mineral nutrient from the medium was reduced at high gelling agent concentration. Gelrite has been reported to yield better results than agar by Henderson, 1987; Van *et al*., 1991 and Welander *et al*., 1992, in the process of regeneration and shoot multiplication. In addition to this, it was reported that agar from different sources contains various amounts of contaminants, whereas phytagel is free from phenolic compounds but has higher ash content than agar (Scherer *et al*., 1988). This may be one of the reasons in the present study the Gelwell containing medium, showing enhanced growth parameters than Agar agar.

**ACKNOWLEDGEMENT**

The authors wish to express their gratitude to the Principal, Perunthalaivar Kamaraj Krishi Vigyan Kendra, Puducherry for providing Lab. facilities to carry out the study. They are also thankful to HOD and other staff members, Department of Botany, Kanchi Mamunivar Centre for Post Graduate Studies, Lawspet, Puducherry for facilities.

**TABLE 1:** Effect of gelling agents on shoot multiplication in Banana var. Poovan grown on MS medium containing 2.0 mg/$L^{-1}$ BAP and 3.0% sucrose

| Treatment | Shoot development (%) | No. of shoot/culture | Shoot length (cm) | No. of leaves/shoot |
|---|---|---|---|---|
| Agar agar (T1) | 100 | 2.22 | 1.78 | 2.84 |
| Gelwell (T2) | 100 | 4.01 | 3.96 | 3.75 |
| CleriGel (T3) | 100 | 2.53 | 2.27 | 3.01 |

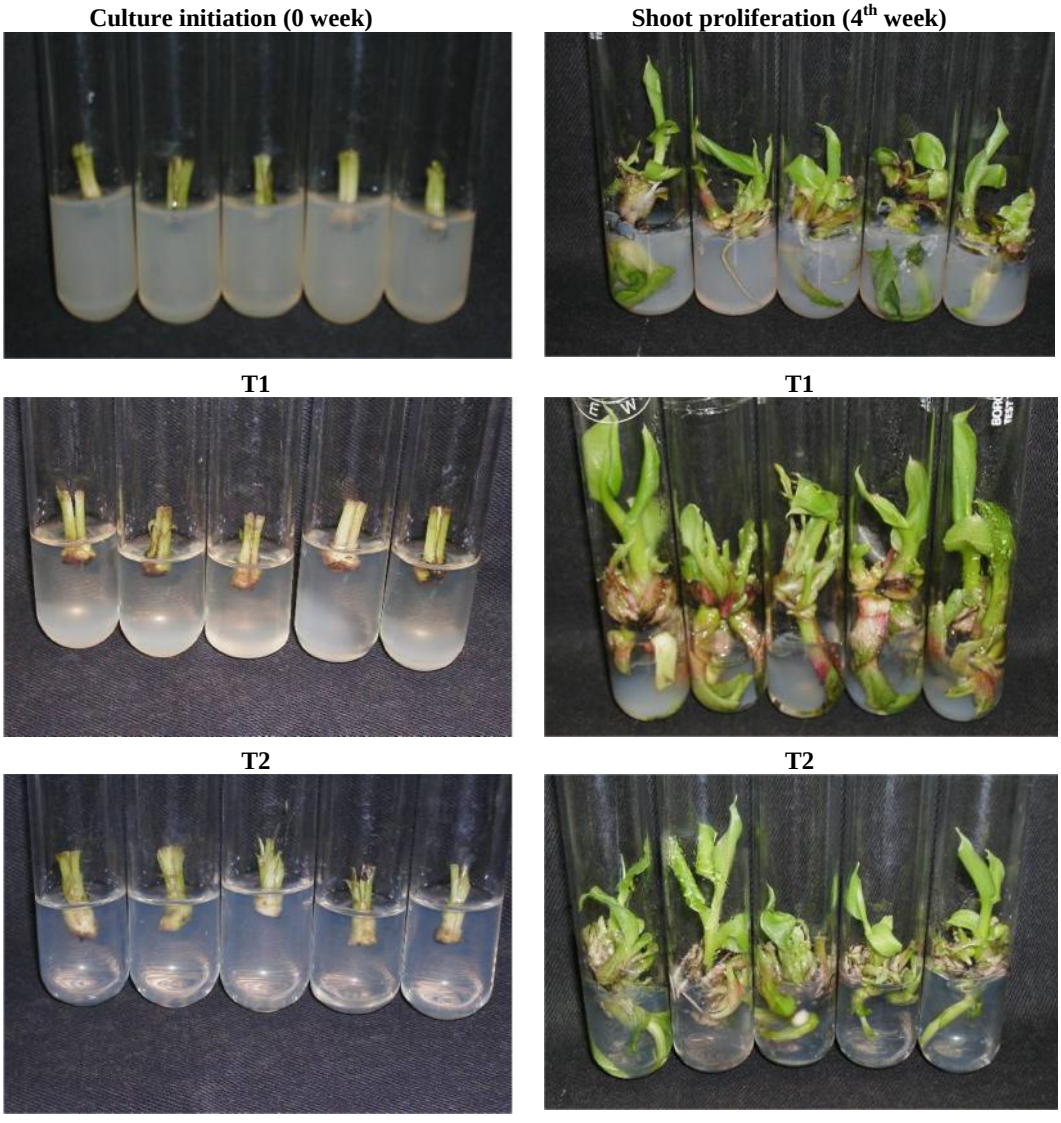



**FIGURE 2:** Banana shoots var.Poovan cultured on three Gelling agents
[ T1 – Agar agar T2 – Gelwell T3 – CleriGel ]

## REFERENCES

Singh, H.P. (2008) R and D in banana and plantain – national and international scenario, *Indian Horticulture*, **53**, No.5, pp.3-5.

Cronauer, S.S., and Krikorian, A.D. (1984) Multiplication of Musa from several excised stem tips. *Annals of botany* **53**: 321-328.

Wong, W.C. (1986) *In vitro* propagation of banana *Musa* spp: Initiation proliferation and development of shoot tip cultures on defined media .*Plant cell tissue and organ culture* **6**:159-166.

Nandwani, D., Zehr, U., and Barwale, R.B. (2000) Mass propagation and ex vitro survival of banana cv.Basarai through tissue culture, Gartenbauwisserschaft, **65** (6): S 237-240.

Kodym, A., and Zapata-Arias, F.J. (2004) Low cost alternatives for the micropropagation of banana, *Plant cell, Tissue and organ culture* **66:** 67-71.

Mohammad, A., Hussain, I., Navqvi, S., and Rashid, H. (2004) Banana plantlet production through tissue culture, *Pak.J.Bot*. **36** (3): 617-620.

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

Kaçar, Y.A., Biçen, I., Varol, Y.Y., Mendi, S., Serçe and Cetiner (2010) Gelling agents and culture vessels affect in vitro multiplication of banana plantlets. *Genetics and Molecular Research* **9** (1): 416-424.

Ramesh, Y., Ramanujam, V., Ramassamy, V. (2014) Effect of gelling agents and Carbon sources in *in vitro* multiplication of banana *(Musa paradisiaca* L.) var. Robusta, *International Journal of Advanced Biological research*, Vol.**4**(2): 153-157.

Kumar, A., Palani, L.M.S., and Nandi, S.K. (2003) The effect of light source and gelling agent on micropropagation of *Rosa damascena* Mill. and *Rhynchostylis retusa* (L.) Bl, *Journal of Horticulture Science and Biotechnology* **78**(6): 786-792.

Debergh, P.C. (1983) Affect of Agar agar brand and concentration on the tissue culture medium, *Physiologia Plantarum* **59**: 270-276.

Romberger, J.A., Tabor, C.A. (1971) The *Picea abies* shoot meristem in culture, 1.Agar and Autoclaving Effects. *American Journal of Botany* **58**: 131-140.

Nairn, B., Furneaux, R.H., Stevenson, T.T. (1995) Identification of an agar constituent responsible for Hydric control in micropropagation in radiata pine. *Plant cell, tissue and organ culture* **43**: 1-11.

Bornman, C.H., and Vogelman, T.C. (1984) Effect of rigidity of gel medium on benzyladenine-induced adventitious bud formation and vitrification *in vitro* in *Picea abies*. *Physiologia Plantarum* **61**:505-512.

Singha, S., Townsend, E.C., and Oberly, G.H. (1985) Mineral nutrient status of crabapple and pear shoots cultured *in vitro* on varying commercial agars. *J.Am.Soc.Hort.Sci*. **110**: 407-411.

Ghashghaie, J., Brenckmann, F., and Saugier, B. (1991) Effect of agar concentration on the water status and growth of rose plants cultured *in vitro*. *Physiologia Plantarum* **82**: 73-78.

Henderson, J.M. (1987) The use of gelrite as a substitute for agar in medium for plant tissue culture. *Alabama Agril*. 2: 5-6.

Van Ark, F.M.A., Zaal, J., Cremers-Molenaar (1991) Improvement of the tissue culture response of seed derived callus cultures of Poa protensis L.; effect of gelling agent and abcisic acid. *Plant cell, Tissue and organ culture* **27**: 275-280.

Welander, M., and Maheswaran, G. (1992) Shoot regeneration from leaf nodes of dwarf apple rootstocks. *Journal of Plant Physiol*. **140**: 223-228.

Scherer, P.A., Muller, E., Loppert, H., and Wolff, G. (1988) Multi-element analysis and gelrite Impurities investigated by inductively coupled plasma emission spectrometry as well as physical properties of tissue culture medium prepared with agar or the gellan gum Gelrite. *Acta Horti*. **226**: 655-658.