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Original Research Article

Influence of Gelling Agents in Micropropagation of Banana var. Grand Naine

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Abstract

This study was aimed to examine the effects of different kinds of gelling agents in micropropagation of banana, *Musa paradisiaca* var. Grand Naine. Four types of gelling agents namely Agar agar (Himedia), Crude agar (NR Chem), 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 with 5.75 shoots per culture, while media containing Agar agar, Crude agar and CleriGel resulted in 5.12, 4.58 and 4.37 shoots per culture respectively. The plantlet length (9.7 cm) was the highest in Gelwell followed by Crude agar (7.9 cm), Agar agar (6.8 cm) and CleriGel (5.8 cm).

Keywords

Agar agar

CleriGel

Crude agar

Gelwell

MS- 230

Introduction

Banana is one of the most important major fruit crop grown in India. The banana culture in India is as old as Indian civilization. It is so predominant and popular among people that poor and rich like the fruit. Considering the year round availability of fruits, unlike the seasonal availability of other fruits, it has become an inevitable necessity in any household in India, in all functions. In conventional field propagation, the production of suckers is highly season dependent and hence, availability of planting material in a given season is often a limiting factor. The planting season in most of the banana production areas starts with the onset of monsoon, which creates a heavy demand for the planting material often leading to supply of sub standard material. Through *in vitro* micropropagation method, the production of planting material can be achieved as per needs (Singh et al., 2011). The growth and multiplication of *in vitro* shoots are affected by many factors (Israeli et al., 1996), including the chemical and physical properties of the culture medium. One of the most important factors which affect the chemical and physical characteristics of the culture medium *in vitro*, is the type and concentration of gelling agent.

Gelling agents have a strong effect on the growth and development of various explants (Romberger and Tabor, 1971; Stoltz, 1971; Werner and Boe, 1980). In addition to the mineral nutrient concentration, the physical properties of the medium, such as water potential and nutrient availability are also affected by the gelling agent (Singha et al., 1985; Kusumoto, 1980). The *in vitro* response of plantlets to gelling agent has been reported to depend on plant species (Singha, 1982). Several studies have been done on the effects of gelling agents on the chemical composition of the growth medium with some plant species (Stoltz, 1971; Werner and Boe, 1980).

Thus in this study, we examined the effects of four types of commercial grade gelling agents on the growth and multiplication of banana shoots cultured on it.

Materials and methods

In vitro stock cultures

Banana, *Musa paradisiaca* var. Grand Naine (AAA) cultivated in Tamil Nadu and Puducherry regions belongs to Cavendish group used for dessert purpose. *In vitro* shoot cultures of banana var. Grand Naine which were maintained at Tissue Culture Laboratory, Perunthalaivar Kamaraj Krishi Vigyan Kendra, Puducherry, South India, were used to generate the stock cultures as source of explants for the present study. These shoots were repeatedly sub-cultured on MS-230 semi-solid medium at an interval of 4 weeks until sufficient stock of explants was available for initiating the experiment.

Media preparation

MS basal medium (Murashige and 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 Crude agar (T2), 2 gm/litre CleriGel (T3), Gelwell (T4) 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-T4) were then autoclaved at 121°C for 20 min.

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-T4) by keeping the corm base completely immersed in the medium. 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°C. Average of 10 cultures / treatment was taken for number of shoots and number of leaves at the end of fourth week.

Results and discussion

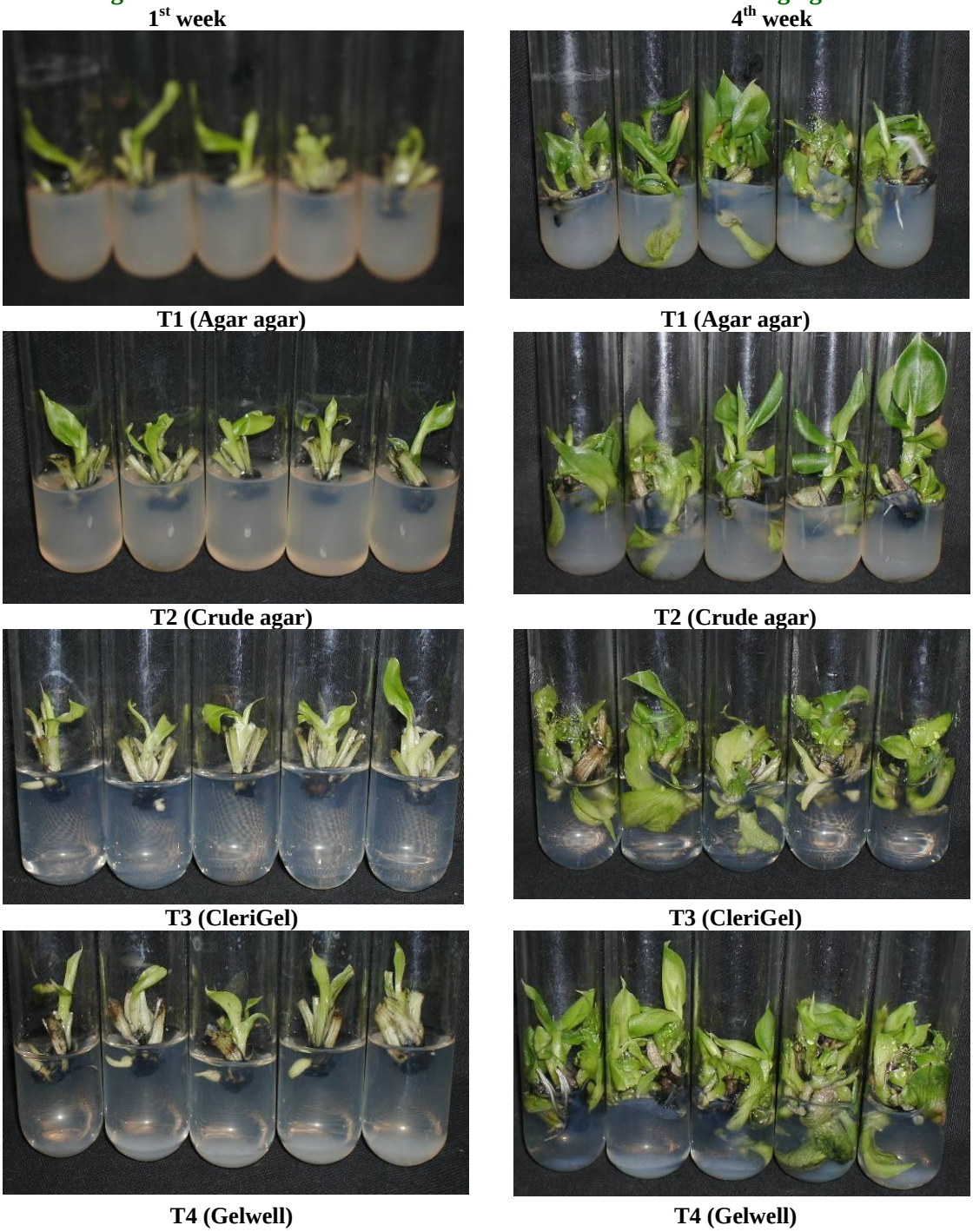
The effects of four commercial grades of gelling agents (Agar agar, Crude agar, CleriGel, Gelwell) were compared in the present study. Their effects on *in vitro* multiplication of banana var. Grand Naine had a significant changes on the parameters studied such as number of shoots, plantlet height and number of leaves/plantlet after acclimatization (Fig. 1).

Number of shoots: 100% shoot development was observed in all the four treatments carried out. But the number of shoots per culture was affected by gelling agent added. Gelwell increased the number of shoots (5.75/culture) followed by Agar agar (5.12/culture), Crude agar (4.58/culture) and CleriGel (4.37/culture) (Table 1).

Table 1. Effect of gelling agents on shoot multiplication in Banana var. Grand Naine grown on MS medium containing 2.0 mg/L^{-1} BAP and 3.0% sucrose.

Treatment	No. of shoots / culture	No. of leaves / plantlet	Length of plantlet (cm)
T1	5.12	4.7	6.8
T2	4.58	5.2	7.9
T3	4.37	4.5	5.8
T4	5.75	5.7	9.7

Fig. 1: Banana shoots var. Grand Naine cultured on four Gelling agents.



Length of plantlet: The cultures derived from Gelwell showed enhanced plantlet length (9.7 cm) than the shoots derived from Crude agar (7.9 cm), Agar agar (6.8 cm) and CleriGel (5.8 cm) (Table 1) (Fig. 1).

Number of leaves: Number of leaves per plantlet varied significantly in different gelling agents used in the present study. It was greater in Gelwell (5.7) followed by Crude agar (5.2), Agar agar (4.7) and least in CleriGel (4.5) (Table 1) (Fig. 1).

It was found from the present study that Gelwell (Microxpress) which was stated equivalent to Phytagel has beneficial effect on shoot multiplication and growth in the micropropagation of banana var. Grand Naine. 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 three commercial grade gelling agents on *in vitro* multiplication of banana var. Poovan reporting that gel medium (Gelwell) 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 and Tabor, 1971), impurities (Nairn et al., 1995) and gelling strength (Debergh, 1983).

Choice of gelling agent can have a strong effect on growth and development of plant tissue cultures (Tremblay and Tremblay, 1991). Among agars, the brand used can also cause differential results. These effects may be the result of differences in the strength of the gel produced, mineral composition and/or availability, and the presence of inhibitory compounds (Scholten and Pierik, 1998).

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 Ark 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.

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