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A Comparison of *In vitro* Mass Propagation of Kew with Mauritius Type of Pineapple (*Ananas comosus* Merr L.)

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ABSTRACT. *Ananas comosus L. Merr (Pineapple) is conventionally propagated through suckers, slips and ratoons taken from existing cultivations. These planting materials are not homogeneous and this is a major difficulty that farmers face in large scale cultivations. In vitro micropropagated plants offer a possible answer due to their high level of uniformity. As propagation of Mauritius type, through in vitro meristem culture is already established, a similar technique for mass propagation of Kew type (Smooth Cayenne) was compared in this study. Shoots were proliferated on Murashige and Skoog (1962) liquid and semi-solid media, supplemented with benzyl amino purine (BAP, 2.5 µmol) and indole acetic acid (IAA, 1.25 µmol). A higher proliferation was detected in liquid than in semi-solid media, but was less than that of Mauritius type. Plants were successfully regenerated on BAP (1.25 µmol) and IAA (1.25 µmol) semi-solid media. Similar results were observed on Agar (BDH) and unpurified moss jelly as the solidifying agent, suggesting that agar could be replaced with less expensive moss jelly. Plants rooted on MS with indole butyric acid (IBA, 1.25 µmol) resumed independent growth after eight weeks of acclimatization in the green house.*

INTRODUCTION

There are two main types of *Ananas comosus* L. Merr. (Pineapple, Family: Bromeliaceae) cultivated in Sri Lanka: Kew (Smooth Cayenne) type and Mauritius (Queen) type. Until recently, pineapple was cultivated on a small scale for local consumption and the local market is yet for Mauritius type. The type Kew (Smooth Cayenne) has a better commercial value when grown for processing and fresh fruit markets. With the realization of increased export potential for the Cayenne type, cultivation has been initiated in several hundred hectares and the growers are interested in this type.

Sucker production of Kew type is poor, thus the planting material obtained from existing cultivations is lower than that of Mauritius. They are also not homogeneous. The major constraint therefore in cultivation is to

obtain sufficient number of homogeneous planting material. Micropropagation through meristem culture, offers a solution.

Several reports are available on *in vitro* culture of Mauritius type pineapple (Sita Lakshmi *et al.*, 1974; Mathews *et al.*, 1976; Fernando, 1986; Hirimburegama and Wijesinghe, 1992). The present study was therefore carried out (i) to establish a protocol for *in vitro* propagation of Kew type, (ii) to compare with that of the Mauritius type, (iii) to test the multiplication rate in liquid and semi-solid media and (iv) to examine the effect of unpurified locally manufactured agar on *in vitro*.

MATERIALS AND METHODS

Shoot meristems of young ratoon suckers (1-2 months of age) of Kew and Mauritius types collected from the field were used as the explant. Most leaves, were removed from the suckers, thoroughly washed with soap (Sunlight ™) and were surface sterilized by immersing in 70% alcohol (v/v; 96% commercial grade) for 10-15 min followed by 15% Clorox (a commercial bleach, 5.25% NaOCl) for 20 min. Under sterile conditions, the white shoot tip (apical meristem together with 2-3 leaf primordia) was obtained and inoculated on culture medium as explained below.

Culture medium and conditions

Murashige and Skoog, (1962), basic mineral nutrients (MS) was supplemented with BAP (2.5 μmol) and IAA (1.25μmol; Hirimburegama and Wijesinghe, 1992) and solidified with 0.6% agar (BDH grade). In a similar experiment, agar was replaced with locally available moss jelly (Marina Moss Jelly ™) at the same concentration. The liquid media contained the above combination of growth regulators without agar. After adjusting the pH to approximately 5.8, they were autoclaved at 102kPa and 121°C for 20 min. Cultures were incubated at 28±1°C and 60% RH at a 16h photoperiod provided with Osram cool white fluorescent tubes. Liquid cultures were incubated under continuous shaking at 60 rpm on an Orbital shaker.

Maintenance of proliferating shoots

When 10-15 shoots were produced by single shoot meristems, they were separated into 2-3 pieces each containing 3-4 shoots and were

inoculated on to a similar freshly prepared medium. Cultures were maintained by subculturing in this manner in every 6 weeks.

Plant regeneration, rooting, acclimatization and field testing

Proliferating shoots (height 3cm) containing about 6 leaves were placed on MS medium (1962) containing BAP (1.25μmol) and IAA (1.25μmol), for shoot development. Plant regeneration was tested on agar (BDH) as well as on Moss jelly™. The medium was solidified with 2.5g of moss jelly per 100cm^3.

Regenerated shoots (4 weeks on regeneration medium) were placed on MS medium containing IBA (1.25μmol) for root development. After 4 weeks, rooted plants were transferred to pots (sand : soil at a ratio of 1:1) and kept in the green house under humid conditions. After 8 weeks of acclimatization, plants were transferred to the field.

The number of shoots produced over time on liquid and semi-solid conditions, plant height, and the number of leaves and roots during regeneration were determined. Time taken for the onset of fruits (both in Mauritius and Kew) was also noted.

Statistical analysis was done using Tukey's HSD test at 5% level of significance. The average value of 50 replicates was used in statistical analyses.

RESULTS AND DISCUSSION

Single meristem tips of both Kew and Mauritius type proliferated producing multiple shoots, but the rate was higher in Mauritius type (Figure 1). However, the study confirmed the previous observation of Hirimburegama and Wijesinghe, (1992) that shoot tips of *Ananas comosus* L. Merr, could be successfully proliferated *in vitro* in the presence of IAA (1.25μmol) and BAP (2.5μmol). In proliferation, apart from the apical meristem, pre-existing dormant axillary meristems may also have contributed, especially due to the presence of cytokinin (Hirimburegama and Gamage, 1995). Removal of excessive leaf scales around the meristem enhanced shoot (meristem) proliferation in pineapple. This enhancement is probably due to the exposure of shoot and axillary meristems to the effect of growth regulators.

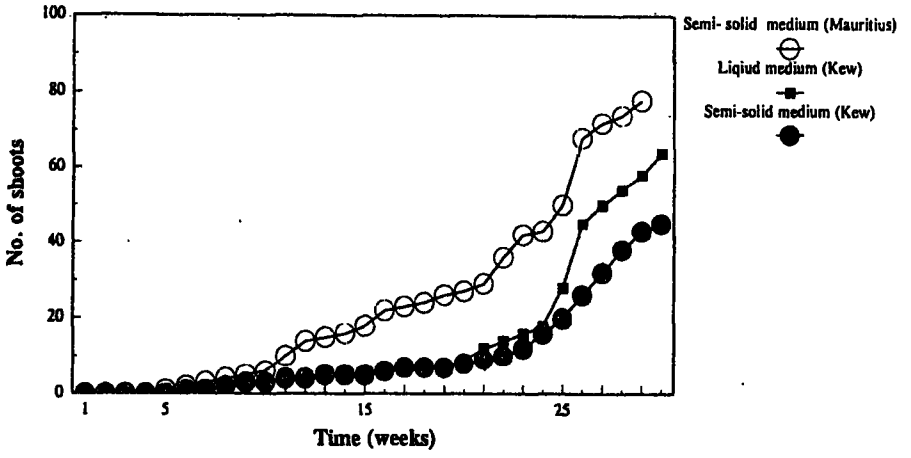

Figure 1. The rate of shoot proliferation in liquid and semi solid medium by Kew and Mauritius type of pineapple.

Comparison of shoot proliferation in liquid and agar

Shoot proliferation was low at the beginning (Figure 1). However, it increased rapidly in both liquid and semi-solid media from the 20th week. A significant increase in shoot number was seen after 25 weeks at the time of first sub-culturing. A significant enhancement of shoot multiplication was observed after initial subculture. The number of shoots in the liquid media was significantly greater than in the semi-solid media in second and third subcultures (Table 1).

Table 1. Shoot production (average no. of shoots in 50 replicates) in successive subcultures in liquid and semi-solid media of Kew type.

Medium	S_0	S_1	S_2	S_3
Semi-solid	7.2 ± 0.4^{ab}	15.0 ± 0.9^{b}	40.2 ± 5.5^{c}	95.6 ± 4.7^{e}
Liquid	5.8 ± 0.6^{ab}	16.2 ± 1.7^{b}	69.4 ± 4.4^{d}	128 ± 11.2^{f}

S_0, S_1, S_2, S_3 refer to the initial, first, second and third subcultures respectively. Comparisons were done using Tukey's HSD test at 5% level of significance. Values followed by the same supercript are not significantly different.

Shoot proliferation improved when the newly formed leaves of the shoot tip were removed. This was also true for proliferating shoots. In both pineapple types, subculturing enhanced shoot multiplication, where it was significantly greater with time (Table 1).

Plant regeneration on moss jelly and agar

In vitro plants regenerated equally well, on jelly moss and agar (BDH grade) in the presence of 1.25μmol IAA and 1.25μmol BAP (Figure 2 a-c).

There was no significant difference in growth of plants regenerated on the two solidifying agents (Figure 2 a-c). When the plants were transferred to a medium with IBA (1.25μmol), a well developed root system was obtained within 3-4 weeks, on moss jelly and agar.

When planted in the field true-to-type plants were developed both in Kew and Mauritius types. The onset of fruits was observed at 16 months after transfer to the field. In both types, the fruits were morphologically true to type.

Genetic off types in pineapple were reported when the multiplication stage involved a callus (Drew, 1980). A high level of cytokinin in the multiplication medium is also known to favour induction of somaclonal variation (Larkin and Scowcroft, 1981). However, use of 5μmol BAP for multiplication in banana resulted in a minimum production of off types (Cronauer and Krikorian, 1984). In the present study, a concentration lower than 5μmol BAP was used and did not involve a callus stage. Therefore, off types were low.

The study confirms the suitability of liquid cultures over semi-solid media for rapid shoot proliferation in Kew pineapple. Enhancement of shoot proliferation in liquid cultures has also been reported in some other crops (Ziv, 1991). This could be attributed to continuous aeration, causing equal distribution of nutrients in the medium. The number of shoots proliferated in liquid and semi-solid media showed a significant increase between the second and the third subcultures (Table 1). With successive subcultures, shoots proliferated with very few leaf primordia. All the above can be considered as the effects of cytokinin.

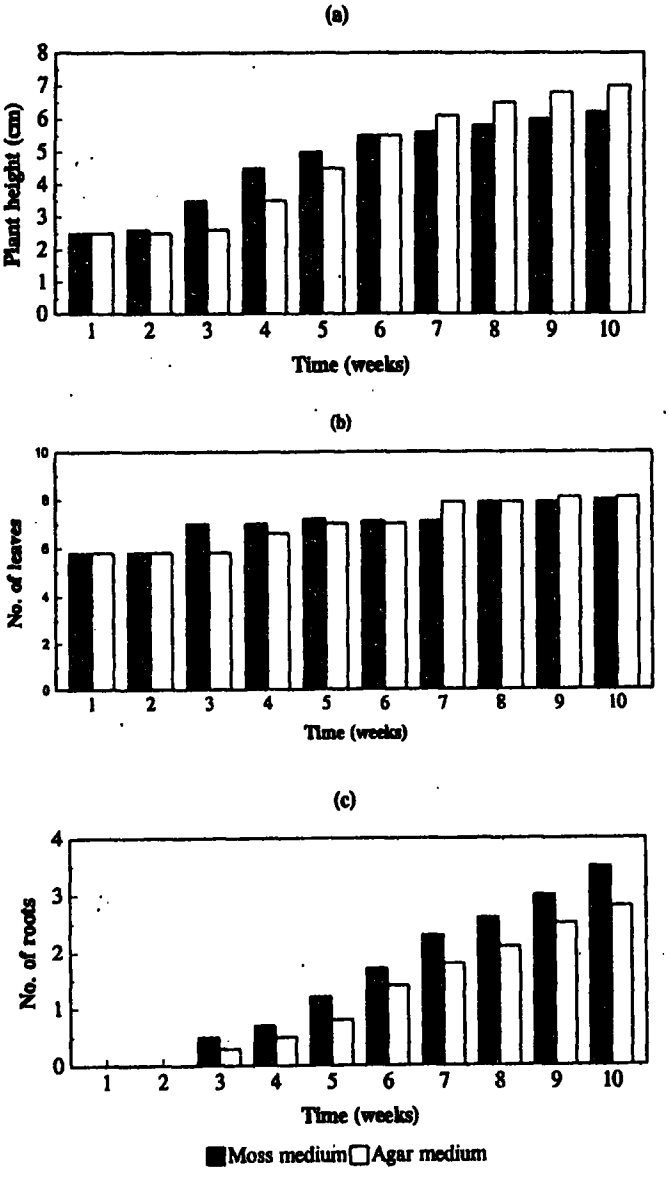

Figure 2. **A comparative study on *in vitro* growth of pineapple type Kew on agar and moss jelly. (a) The effect on plant height, (b) The effect on the number of leaves, (c) The effect on the number of roots.**

Another significant outcome of the study was the successful use of locally available jelly moss. Eventhough there was no significant difference in plant growth on agar and jelly moss (Figure 2a-c), physical vigour of plants on jelly moss appeared to be better. Root initiation was better in moss media (Figure 2c), probably due to its watery nature and impurities as reported by Pierik (1991). Agar, an extract from sea weeds, is a classical gelling agent used in plant tissue culture. Although a natural product, its high cost may be due to purification involved. Therefore, in developing countries like Sri Lanka, use of this purified agar in commercial scale makes the end product expensive. Use of moss jelly would reduce the cost per plant to a great extent. This would improve the practical applicability of the technology.

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