In Vitro Performance of Hybrid Pineapple (*Ananas comosus* L.)

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ABSTRACT

A series of hybrid pineapples has been produced for the first time in Sri Lanka by the Department of Agriculture. These hybrids are still under research level and *in vitro* characteristics that are useful in micropropagation are yet to be identified. Therefore, an experiment was conducted to study the *in vitro* shoot initiation ability of two hybrid pineapples; H1 and H3, at different 6-benzyleaminopurine (BAP) levels (0, 0.5, 1.0, 1.5, 2.0 and 2.5 mgl^{-1}) in Murashige and Skoog (MS) medium. It was revealed that 1.5mgl^{-1} 6-benzyleaminopurine in MS medium was the optimum level for shoot initiation of H3 while H1 did not show variation in shoot initiation at tested hormone levels.

KEYWORDS: 6-Benzyleaminopurine, Hybrid Pineapple, *In Vitro* Performance.

INTRODUCTION

Pineapple is a tropical fruit which is very popular all over the world. It is the third most important tropical fruit in the world (Robrbach *et al.*, 2003). In Sri Lanka, pineapple has now become the second most demanded fruit among consumers (Anon, 2003). Mauritius, a clone of cultivar Queen, is the most popular pineapple type among Sri Lankan farmers. It is a sweet pineapple type and mainly consumed as a fresh fruit. In world context, the most popular pineapple type is Kew, a clone of cultivar Smooth Cayenne. It accounts for about 70% of world pineapple production and about 95% of canned pineapple is manufactured using this cultivar (Robrbach *et al.*, 2003). Exportation of pineapple as a source of foreign exchange to Sri Lanka is hindered to a great extent due to the presence of sharp spines on leaves and production of small conical shape fruits of Mauritius which limits its use as a row material in pineapple processing industry. However, the increasing demand for fresh pineapple in the temperate markets is putting pressure on producers to cultivate cultivars that are superior to Smooth Cayenne as a fresh fruit since Smooth Cayenne does not provide present consumers with the best fruit quality (Paul, 1993; Sanewski and Scott, 2000). These trends in the world have created an opportunity for pineapple growers to produce more and more sweet taste types such as Mauritius.

To make use of this opportunity, the Department of Agriculture, Sri Lanka, has launched a program aiming at the production of improved pineapples through genetic modifications and have produced hybrids namely H1 and H3 using mother stocks of Mauritius and Kew types. In H1: spines are present only on the distal 1/3 of the leaves, no spines on crown leaves, eyes are prominent, fruit is cylindrical and taste is sweet, where as in H3: spines are present only at the tip of the leaves, no spines on crown leaves, eyes are not prominent, fruit is barrel shaped, taste is in between Mauritius and Kew. Other than the above improved characteristics, these hybrids give an average yield of about 30 tons per hectare which is 200 percent higher than that of standard Mauritius and they also show resistance against pineapple wilt to a certain extent (Alwis, 2005).

Since the conventional vegetative propagation methods cannot meet the demand of planting material in commercial level, *in vitro* propagation is suggested as an alternative to planting material production of pineapple (Smith *et al.*, 2003). *In vitro* performance of a plant greatly depends on its genetic composition. As the genotypes of hybrids are different, *in vitro* performance of H1 and H3 may differ from that of standard Mauritius and Kew. Therefore, it is necessary to study the performance of pineapple hybrids in different stages of *in vitro* culture to introduce a protocol for *in vitro* planting material production of pineapple hybrids.

MATERIALS AND METHODS

This experiment was carried out at the Regional Agricultural Research and Development Center (RARDC) of the Department of Agriculture at Makandura (NWP) Sri Lanka, from February to June 2005.

Suckers of about 6 inches long were obtained from hybrids (H1 and H3) and standard Mauritius type. The plants were decapitated and maintained under 18.2mm average weekly rain fall, and 32^{0}C monthly average temperature in an open environment. Ninety six suckers from each category were used for the preparation of explants. All the leaves, spirally arranged surrounding meristem were carefully removed, and the last two to three leaves were kept intact. The stem portion and all the roots were removed. Foreign matters present on the explants were removed using clean water. Afterwards, the explants were washed in running water for one hour followed by a solution of liquid soap three times and transferred to laminar air flow cabinet. The explants were dipped in 0.5gl^{-1} topsine (Thiophanate methyl 70%, J.L. Morison Son and Jones, Ceylon Ltd.) solution for twenty minutes, followed by 0.5gl^{-1} streptomycin solution (Streptomycin sulphate, Sigma Aldrich Company Ltd.) for another twenty minutes. Later the

explants were dipped in 10% Clorox (5.25% Sodium hypochlorite) solution for fifteen minutes. The explants were then washed 3 times in sterilized distilled water. The size of explants was reduced to make them 0.5cm^3 cubes with a meristem each. The prepared explants were cultured on Murashige and Skoog medium (MS) (Murashige and Skoog, 1962) supplemented with 100mgl^{-1} myoinositol, 30gl^{-1} sucrose, 15gl^{-1} agar and 6-benzyleaminopurine (BAP) (0, 0.5, 1.0, 1.5, 2.0 and 2.5 mgl^{-1}).

The cultures were incubated under 16 hour photoperiod, 25C^0±1 temperature and 75% relative humidity. All possible efforts were taken to minimize the contaminations in each and every step of the experiment. The experiment was arranged in a Completely Randomized Design. Number of days taken for initiation of shoots was recorded. Data were analyzed using SAS computer software (SAS, 1990).

RESULTS

Effect of different BAP levels on shoot initiation of pineapple hybrid H1

Number of days taken for shoot initiation in explants of hybrid H1 varied from 8 to 10 days. Minimum number of days (8.1 days) for shoot initiation was observed at 1mgl^{-1} BAP and maximum number of days (10.9days) was observed at 0mgl^{-1} BAP. Time required for shoot initiation of explants cultured on MS medium treated with 0.5, 1.5 2.0 and 2.5mgl^{-1} BAP were 9, 8.6, 10.1 and 9.6 days respectively. There was no significant difference in the number of days required for shoot initiation of H1 at tested BAP levels (Table1).

Table 1. Mean number of days taken for shoot initiation in pineapple hybrid H1

BAP (mgl^{-1})	Mean number of days
0	10.9 [a]
0.5	9.0 [a]
1.0	8.1 [a]
1.5	8.6 [a]
2.0	10.1 [a]
2.5	9.6 [a]

[a] Mean values with unlike superscripts are significantly different ($p < 0.05$).

Effect of different BAP levels on shoot initiation of pineapple hybrid H3

Number of days taken for shoot initiation in explants of hybrid H3 varied from 8 to 14 days showing a significant difference in the tested hormone levels. 1.5mgl^{-1} BAP at which shoot initiation observed in 8.4 days was the most effective hormone level for H3 hybrid. The highest number of days required for shoot initiation (14.2 days) was recorded when MS medium was treated with 2.5mgl^{-1} BAP. When the medium was treated with 0, 0.5, 1 and mgl^{-1} BAP, shoot initiation was recorded in 11.6, 10.1, 8.6 and 13.3 days respectively (Table 2).

Table 2. Mean number of days taken for shoot initiation in pineapple hybrid H3

BAP (mgl^{-1})	Mean number of days
0	11.6 [ad]
0.5	10.1 [ac]
1.0	8.6 [bc]
1.5	8.4 [c]
2.0	13.3 [af]
2.5	14.2 [def]

[a,b,c,d,e,f] Mean values with unlike superscripts are significantly different ($p < 0.05$).

Effect of different BAP levels on shoot initiation of standard Mauritius

Shoot initiation of standard Mauritius showed significant differences among different BAP levels. The minimum number of days required for shoot initiation (7.1 days) was observed at 0.5mgl^{-1} BAP while the maximum number of days (14 days) was observed at 1mgl^{-1} BAP level. Average number of days, 7.3, 10.9 and 13.6 were observed at 0, 2.5 and 1.5mgl^{-1} BAP levels respectively (Table 3).

Table 3. Mean number of days taken for shoot initiation in standard Mauritius

BAP (mgl^{-1})	Mean number of days
0	7.3 [a]
0.5	7.1 [a]
1.0	14 [b]
1.5	13.6 [b]
2.0	11.8 [b]
2.5	10.9 [b]

[a,b] Mean values with unlike superscripts are significantly different ($p < 0.05$).

DISCUSSION

H1 did not show a significant difference in shoot initiation among tested BAP levels. However, use of hormones at establishment stage has a great effect on plant development in later stages of *in vitro* process, and the use of BAP in MS medium is essential for the regeneration of plants from shoot apices of pineapple (De Almeida *et al.*, 2002).

1.5mgl^{-1} BAP was the best level for shoot initiation of H3. Many authors have described that 1.5mgl^{-1} BAP is the optimum hormone level for shoot initiation of pineapple and it is compatible with the results of this experiment. However, the number of days taken for shoot initiation in H3 hybrid at 1.5mgl^{-1} BAP was not significantly different from those at 0.5 and 1.0mgl^{-1} BAP.

At relatively high concentrations of BAP (2.0 to 2.5mgl^{-1}), the shoot initiation was significantly lower than that of the best level of 1.5mgl^{-1}. Higher mean number of days required for shoot initiation of H3 at 2.0 and 2.5mgl^{-1} BAP may have been due to the detrimental effects of higher BAP on shoot generation (De Almeida *et al.*, 2002).

Standard Mauritius, used as a control in this experiment gave deviated results. According to De Almeida *et al*, (2002), 1.5mgl^{-1} BAP is the optimum level for the establishment stage of pineapple. However, in this experiment 0 and 0.5mgl^{-1}

concentrations of BAP resulted the quickest shoot initiation.

CONCLUSIONS

There is no variation in time required for shoot initiation in bud apices of H1 among the tested BAP (0, 0.5, 1.0, 1.5, 2.0, and 2.5mgl^{-1}) levels. H3 hybrid showed a variation in number of days required for shoot initiation and the best performance was recorded in 1.5mgl^{-1} BAP. However, the long term effects of the use of BAP in establishment medium was not revealed as the experiment was restricted to a study only on shoot initiation stage. Therefore, further studies should be carried out to investigate the impact of BAP in establishment stage on *in vitro* as well as *in vivo* performance of micropropagated pineapple hybrids H1 and H3.

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