

IN VITRO PROPAGATION OF MAURITIUS PINEAPPLE

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ABSTRACT

Axillary buds from crown and ratoon suckers of *Ananas comosus* cv. Mauritius were established in a solid medium supplemented with MS macro and micro salts, White's vitamins and 1 mg/l BAP. Transference of growing buds to a medium of the same basal composition supplemented with 10% coconut water and 10 mg/l NAA produced vigorously growing shoots with a few roots. Transfer of these shoots to liquid medium with 1 mg/l BAP and 80 mg/l Adenine sulphate and subjected to constant agitation at approximately 70 r. p. m. gave more than 10-fold increase in shoot production within 6 weeks as compared to stationary cultures with filter paper bridges as supports. Individual shoots were rooted on solid medium with 0.5 mg/l IBA. Plantlets were successfully transferred to a medium of sand, coir dust, cattle manure, 1:2:1.

Abbreviations : BAP — Benzyl Amino Purine
CW — Coconut Water
IAA — Indoleacetic acid
IBA — Indolebutyric acid
NAA — Naphthelene acetic acid

INTRODUCTION

Pineapples are propagated vegetatively by suckers, slips or crowns of the ripe fruits. It has been shown that plants could be raised by segment cuttings but the number obtained by this method was limited, 16—25 suckers per adult plant (Kotalawala, 1971). Since the application of tissue culture for rapid clonal propagation has been reported for many economically important plants (Murashige, 1974), this research was undertaken to mass propagate the table variety of pineapple cv. Mauritius in an effort to meet the demand for planting material.

MATERIAL AND METHOD

Lateral buds from the crown and ratoon suckers of pineapple cultivar Mauritius were used as experimental material. The leaves were removed and the condensed stem with exposed buds was kept under running water for half an hour prior to sterilization. This were then subjected to surface sterilization for 20 minutes with 10% chlorox with tween 20 added at the rate of 2 drops per 100 ml of solution. The material was washed three times with sterilized water. The lateral buds were excised and cultured on MS medium (Murashige and Skoog, 1962) with White's vitamins (White, 1963) and 1 mg/1 BAP, solidified by adding 6 g/1 Agar. After 6 weeks in culture the growing buds were transferred to medium of the same basal composition supplemented with 10% CW and 10 mg/l NAA. The single shoots obtained after 4 weeks were transferred to filter paper bridges in liquid medium containing 80 mg/1 of Adenine sulphate and various concentrations of BAP ranging from 0—12 mg/1. Culture tubes of capacity 25 × 150 ml were used in this experiment. Each tube contained 25 ml of liquid medium with a filter paper bridge as support. The test was repeated with BAP concentrations ranging from 0—2 mg/1. The single shoots were then transferred to a flask of capacity 250 ml containing 100 ml of liquid MS medium with 1 mg/1 BAP and 80 mg/1 of Adenine sulphate and subjected to constant agitation of approximately 70 r. p. m. The shoots were subcultured at every three-week interval. Individual shoots were rooted on MS medium with 0.5 mg/1 IAA or IBA. The plantlets were transplanted in a mixture containing sand, coirdust, cattle manure, 1 : 2 : 1.

RESULTS

Axillary buds taken from ratoon suckers gave a higher survival percentage than those obtained from the crown of the ripe fruit. Buds when transferred to MS medium with high concentration of NAA and 10% CW grew into healthy single shoots containing 6—8 leaves in a 4—6 week period (Fig 1). These single shoots when cultured in liquid medium with filter paper bridges as supports containing 0, 1.5, 3.0, 6.0 and 12 mg/1 BAP gave highest number of shoots at 1.5 mg/1. There was no significant difference among 1.5, 3.0 and 6.0 mg/1 BAP (Table 1). However, visual observations showed that shoots produced at 6 mg/1 BAP concentration possessed shorter and curved leaves, as compared to shoots produced at 1.5 and 3.0 mg/1 BAP. When the test was repeated with 0, 0.5, 1.0, 1.5 and 2.0 mg/1 BAP there

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was no significant difference among treatments 1.0, 1.5 and 2.0 mg/l BAP (Table II). Therefore, the lower concentration 1.0 mg./l BAP was considered as the optimum for shoot proliferation. Transfer of single shoots to liquid medium (100 ml of medium in 250 ml conical flasks) with 1 mg/l BAP and 80 mg/l Adenine sulphate and kept under agitation of approximately 70 r. p. m. showed more than 10 fold increase as compared to stationary liquid culture on filter paper bridges (Table III, Fig. II). Shoots produced roots in solid medium with either 0.5 mg/l IAA or IBA. Plantlets were successfully transplanted in a mixture containing sand, coir dust, cattle manure, 1:2:1 (Fig. III and IV).

DISCUSSION

Use of dormant buds in clonal propagation of pineapple has been demonstrated (Mathews *et al* 1976). In our experiments the lateral buds from ratoon suckers showed greater percentage of survival than lateral buds from the crown of the fruit. Transfer of growing buds to a medium with NAA gave vigorously growing single shoots with no callus. Transfer of these single shoots to liquid medium with constant agitation yielded more than 10 fold increase as compared to stationary cultures of the same composition. Survival of plantlets in soil was almost 100%. Rooting was found to be not an essential factor for transplanting in the soil. Shoots without roots showed successful establishment in soil when *in vitro* obtained plantlets were more than 5 cm in height.

Multiple shoot formation in pineapple was found to be non-synchronous. Experiments are in progress to assess the possibility of synchronization of multiple shoot formation.

ACKNOWLEDGEMENTS

The author wishes to thank Mrs. A. I. Wickremasinghe, Experimental Officer and Miss. V. H. K. Premachandra, Research Assistant, for their assistance in maintaining the cultures.

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Table I. Effect of BAP on Shoot Proliferation. MS + 80 mg / l Adenine Sulphate + BAP — Liquid Medium on Filter Paper Bridges

BAP Conc.	Av. Increase of Shoots / Culture
0	0
1.5	6.4 $\pm$ 0.76
3.0	5.65 $\pm$ 0.78
6.0	5.06 $\pm$ 0.80
12.0	3.56 $\pm$ 0.42

Table II Effect of BAP (0 — 12 mg / l) on Shoot Proliferation. MS + 80 mg / l Adenine Sulphate + BAP — Liquid Medium with Filter Paper Bridges.

BAP Conc. mg / l	Av. Increase of Shoots / Culture
0	0
0.5	4.2 $\pm$ 0.61
1.0	6.25 $\pm$ 0.45
1.5	6.58 $\pm$ 0.67
2.0	6.47 $\pm$ 0.8

Table III Effect of Stationary and Agitated Cultures on Multiple Shoot Formation
MS + 80 mg / l Adenine Sulphate + 1 mg / l BAP.
16 Cultures / Treatment Incubated for 42 Days

	Stationary Cultures	Agitated Cultures
% multiple shoot formation	100	100
Total number shoots (16 cultures)	105	1108
Av. number of shoots / culture	6.5 $\pm$ 0.51	73.93 $\pm$ 5.10



Fig. 1. Single shoots produced after 6 weeks in culture MS + 10% coconut water + 10 mg / l NAA



Fig. 2. Multiple shoots after 4 weeks in culture MS + 80 mg / l adenine Sulphate + 1 mg / l BAP

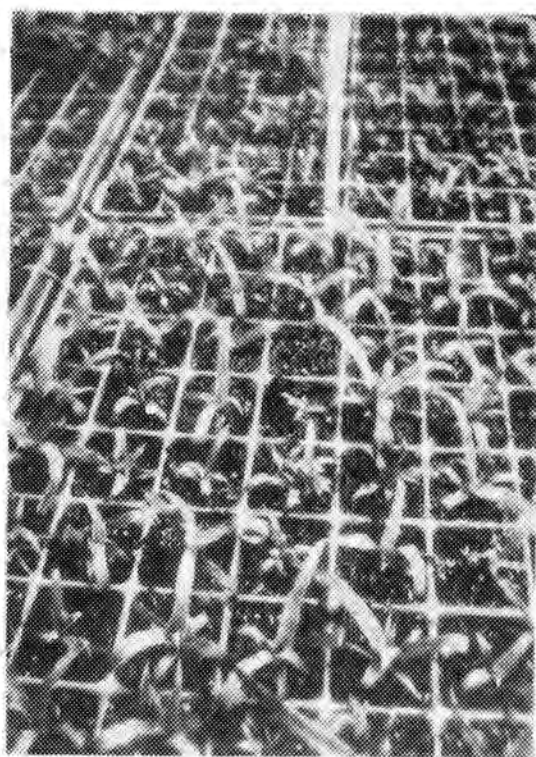


Fig. 3. Plantlets transplanted out in trays of size 18×18 inches



Fig. 4. Four - weeks old plant after transplanting in soil