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Production of pineapple plants in vitro

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Abstract

In vitro culture of pineapple (Ananas comosus) was studied to determine the efficiency of axillary bud culture for rapid propagation of several cultivars. The technique used maximizes the success rate of various steps in the production of pineapple plants. Rapid mass multiplication of plantlets started 9 months after explanting with a significant log phase. The number of plantlets obtained from the culture of a single bud by the thirteenth month ranged from 210 to 380 for 'Perolera'; 300 to 350 for 'PR-1-67'; and 40 to 85 for 'Smooth Cayenne'. The method permits culture of a range of pineapple cultivars. Little morphological variation was observed in young regenerated plants.

Introduction

Pineapple is routinely propagated vegetatively by means of lateral shoots, basal suckers, or crowns. Plant material is often limited, especially for the 80,000 plants/ha needed in the establishment of new plantations, and for the propagation of improved cultivars or newly discovered sports.

In vitro propagation of pineapple was first achieved in 1960 by Aghion and Beauchesne (1960), but it was not until the reports of Lakshmi Sita et al. (1974), Teo (1974) and Mapes (1973) that in vitro production of pineapple was considered as a commercial alternative. Most recent reports (Pannetier and Lanaud 1976, Wakasa et al. 1978, Mathews and Rangan 1979, Drew 1980, Zepeda and Sagawa 1981) deal only with the factors involved in the establishment of axillary buds in culture. They do not consider the number of plants regenerated or the effect of genotype on culture establishment.

The objectives of the present study were to test the technique of axillary bud culture for rapid pineapple propagation using different cultivars and to determine the efficiency of plantlet production on a per bud basis. These data would provide an estimation of explant number, space and time requirements to meet production needs.

Materials and Methods

To test the feasibility of axillary bud culture of the various cultivars of pineapple, Ananas comosus (L.) Merr., 20 buds from each of the following cultivars were explanted: 'Red Spanish', 'Cambray', 'Abaka', 'Valera Amarilla', 'Bumanguesa', 'Brecheche', 'Esmeralda', 'PR-1-67', 'Perolera' and 'Smooth Cayenne'. Because of the potential for large numbers of plantlets to be produced from a single bud, 3 buds representative of each of 3 cultivars were selected and followed through 13 months of subculture to determine plantlet production on a per bud basis. These cultivars were 'PR-1-67', a spiny commercial cultivar grown for fresh fruit and canning in Puerto Rico; 'Perolera', a smooth-leaved cultivar grown in the Northern Andes of South America and used for fresh fruit; and 'Smooth Cayenne', the most widely grown pineapple cultivar in the world.

Crowns and stems were rinsed in water, defoliated and surface-sterilized by agitation in a 20% clorox solution (1% sodium hypochlorite) with 2-3 drops of a surfactant (Tween 20) for 20 min, followed by 3 rinses of 10 min each in sterile water. Terminal and axillary buds were then excised aseptically and surface-sterilized in 2% clorox solution for 10 min followed by three 10 min rinses in sterile water. Buds were explanted in 60 x 15 mm petri dishes or subcultured into test tubes containing Murashige and Skoog (1962) medium, supplemented with 3% sucrose, 0.8% Difco Bacto-agar, 0.57 mM inositol, 1.2 µM thiamine HCl, 10.8 µM NAA and 8.8 µM BA, adjusted to pH 5.7 and autoclaved at 121° C, and 1.1 kg cm^{-2} for 20 min. Cultures were incubated at room temperature (24-27° C) with a 16 h photoperiod of 76 µmol s^{-1} m^{-2} provided by cool-white fluorescent lamps. Explants were subcultured onto fresh medium in test tubes at 6 week intervals until adequate growth had occurred for transfer to liquid medium.

Proliferating explants were multiplied in 50 ml liquid cultures of the same medium in 125 ml Erlenmeyer flasks, maintained at 100 rpm on rotary shakers. Liquid shake cultures were subcultured at approximately 4 week intervals, when flasks had become tightly packed with plantlets. In this way, numerous flasks of callus and regenerating plantlets were obtained from one original bud.

After each subculture, only plantlets 2.5 cm or larger were harvested. They were transferred to individual pots or to flats containing a commercial soil mixture and enclosed in plastic bags, creating a greenhouse effect. The plants were incubated in a growth chamber at 28° C under fluorescent lamps and gradually hardened off by removing the plastic covers. Acclimated plants were transplanted to larger pots and placed in a greenhouse under a black

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Table 1. Mean in vitro pineapple plantlet production per initiated bud.

Month (Subculture)[b]	Mean number of plantlets harvested per flask[a]											
	Perolera			PR-1-67			PR-1-67			Smooth Cayenne		
	Bud 1	Bud 2	Bud 3	Bud 1	Bud 2	Bud 3	Bud 1	Bud 2	Bud 3	Bud 1	Bud 2	Bud 3
9 (7)	2.5	1.0	2.3	1.5	1.7	1.5	0.0	1.7	0.0	0.5	0.0	4.0
10 (8)	2.4	3.3	3.1	3.3	3.5	2.5	3.7	3.8	3.0	0.5	0.5	6.0
11 (9)	3.7	2.8	3.4	3.1	2.7	3.2	3.2	3.2	2.7	2.0	1.3	2.5
12 (10)	6.6	7.5	5.6	5.0	1.0[c]	4.1	6.1	3.8	3.8	3.2	4.8	4.0
13 (11)	12.8	14.8	14.4	11.2	10.5	9.9	6.2	13.8	13.8	4.0	3.8	4.8

[a]Plantlets were larger than 2.5 cm.
[b]Number of months after culture initiation (number of subculturing after inoculation).
[c]Several flasks became contaminated during the experiment.

cloth screen to prevent scorching. The screen was removed 3 weeks later and surviving plants were counted. Fertilization was initially done biweekly with an acid-forming plant fertilizer and, later, with a solution of 10% MS salts to remedy apparent nutrient deficiencies.

The effect of subculturing into basal medium lacking phytohormones was evaluated by inoculating 10 g of an actively dividing 'Perolera' culture into flasks containing 50 ml of basal medium with hormones (10.8 µM NAA and 8.8 µM BA) or without. The two treatments were replicated 9 times.

Results and Discussion

Terminal and axillary buds were excised and placed on solid medium containing auxin and cytokinin to initiate organogenic cultures (Fig. 1A). Multiple buds were initiated from the cultured buds after 2 to 3 months (Fig. 1B), and the cultures could then be transferred to liquid medium to enhance proliferation.

Proliferating cultures were obtained from 75% of the buds explanted, and plantlets were regenerated from all 10 cultivars tested. No plantlets were harvested from cultures during the first 9 months; instead all proliferating material was used to increase culture numbers. Cultures less than 6 months old usually consisted of a mass of short (<2 cm in length), thin shoots. However, by 9 months after initiation cultures exhibited a less compact growth habit and consisted primarily of shoots longer than 2 cm with numerous smaller shoots around their base (Fig. 1C). Studies done using 3 buds from each of 2 plants of 'PR-1-67' and one plant of 'Perolera' and 'Smooth Cayenne' showed that the total number of harvestable plantlets doubled with each monthly subculture after the 11th month (Table 1). Numbers of plantlets that could be obtained by the 13th month from a single bud culture varied from 210 to 380 for 'Perolera'; 300 to 350 for 'PR-1-67'; and 40 to 85 for 'Smooth Cayenne' (Table 2). Approximately 25 plantlets larger than 2.5 cm could be harvested per 125 ml flask at each additional subculture.

This in vitro propagation system was suitable for all cultivars tested. However, in the studies following individual buds, differences were noted between genotypes. 'Smooth Cayenne' was less responsive in vitro than the other 2 cultivars, producing fewer plantlets per subculture (Table 1), and so fewer total plants per initiated bud (Table 2).

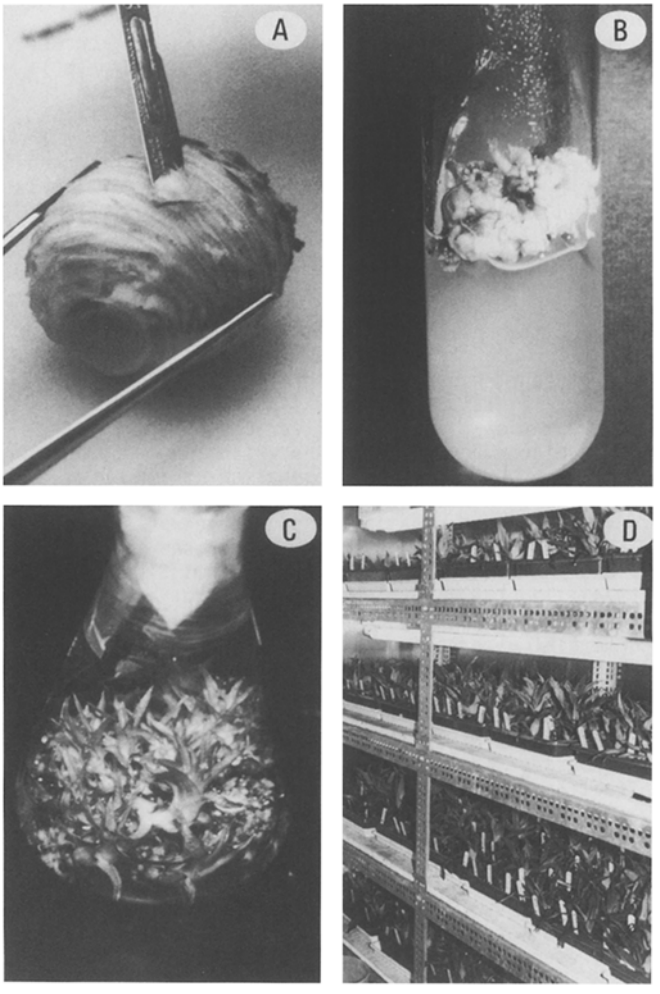

Figure 1. Axillary bud culture of pineapple. A. Buds aseptically removed. B. Proliferating culture 4 months after inoculation of bud. C. Actively dividing culture ready for subculture. D. Regenerated plants.

Table 2. Total numbers of pineapple plantlets produced per initiated bud in five months of harvesting[a]

Cultivar	Bud 1	Bud 2	Bud 3	Totals
Perolera	380	237	212	829
PR-1-67	321	42[b]	344	707
PR-1-67	309	333	112[b]	754
Smooth Cayenne	43	53	85	181

[a]Harvesting began 9 months after culture initiation.
[b]Several cultures became contaminated during the experiment.

Cultures of all 3 cultivars, periodically transferred on multiplication medium with plant growth regulators, exhibited no loss in proliferation capability after 3 years.

Previously reported protocols for pineapple propagation in vitro include a subculture to a medium lacking phytohormones, to induce root formation (Wakasa et al 1978, Mathews and Rangan 1979, Zepeda and Sagawa 1981). In the present research, transferring to a medium without plant growth regulators to induce root formation was not necessary for plantlet survival. Plantlets without roots, produced in multiplication medium with plant growth regulators, had a high survival rate and, while initially smaller than plantlets produced in hormone-free medium, attained the same level of growth after 2 months in soil. In all cultivars, plantlets larger than 3.0 cm had a survival rate of nearly 100% when transferred to soil (Fig. 1D). A 2-fold increase in the total number of harvestable (larger than 2.5 cm) 'Perolera' plantlets was obtained in 6 weeks when plant growth regulators were removed from the multiplication medium (Table 3). However, after 2 subcultures (14 weeks), all plantlets had been harvested and no further growth occurred. Thus, not only was transfer to hormone-free medium not necessary for rooting, it precluded the maintenance of long-term cultures.

There is still one hindrance in the establishment of axillary bud cultures for rapid pineapple propagation. This is the slow growth of, and proliferation from, the initial bud, such that efficient plantlet production is not achieved for 9 months to 1 year after culture initiation. Further experiments need to be done to overcome this obstacle.

Wakasa (1979) reported high levels of variability in regenerated 'Smooth Cayenne' plants. Such variability would compromise the commercial use of in vitro propagation of pineapple, since regenerated plants could not be assured to be clonal in nature. Initial observations of young plants regenerated in these experiments have detected little phenotypic variation; further observations will be made as the plants mature.

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Table 3. In vitro pineapple plantlet production in response to the presence or absence of plant growth regulators in the culture medium.

	Total number of plantlets harvested per flask[a]			
	With plant growth regulators[b]		Without plant growth regulators	
Replicate	1st harvest[c]	2nd harvest[d]	1st harvest	2nd harvest
1	20	23	7	18
2	21	30	9	17
3	16	29	12	21
4	17	25	7	17
5	21	30	11	14
6	27	28	8	16
7	18	21	7	13
8	17	24	6	14
9	20	22	10	18
Totals	177	232	77	148

[a]Plantlets were 2.5 cm or larger.
[b]NAA (10.8 μM); BA (8.8 μM).
[c]Six weeks after inoculation.
[d]Fourteen weeks after inoculation.