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Field performance of tissue-cultured 'Josapine' pineapple

(Prestasi ladang pokok nanas 'Josapine' tisu didik)

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Key words: *Ananas comosus,* pineapple, tissue culture, propagation, somaclonal variation

Abstrak

Anak pokok tisu didik nanas 'Josapine' telah dihasilkan daripada mata tunas jambul. Prestasi ladang pokok tisu didik ini dibandingkan dengan pokok kawalan yang dibiakkan secara penyukuan (quartering).

Sebanyak 12 ciri agronomi telah dinilai iaitu berat buah, berat pokok, berat jambul, panjang buah, lebar buah, saiz empulur, bilangan sulur tangkai, bilangan sulur udara, bilangan sulur bumi, warna isi, jumlah pepejal larut dan nisbah berat buah:pokok. Pokok tisu didik (TC) berbeza dengan ketara apabila dibandingkan dengan pokok yang dibiakkan secara biasa bagi semua ciri kecuali bilangan sulur udara dan warna isi. Pokok TC kurang subur, lebih ringan dan mengeluarkan buah yang kecil. Buah kecil TC mengakibatkan empulur kecil, jambul ringan dan meningkatkan jumlah pepejal larut. Populasi pokok TC juga menunjukkan variasi yang lebih tinggi khususnya bagi semua ciri buah.

Perubahan somaklon TC yang paling ketara ialah pokok berduri (14.7%) dan peningkatan pengeluaran sulur tangkai (hingga bilangan 7). Walau bagaimanapun, ciri pokok TC yang lebih mengkhuatirkan ialah pokok kurang subur dan pengeluaran buah yang saiznya tidak ekonomik. Perubahan secara agronomi diperlukan untuk pengurusan pokok TC supaya dapat mempercepat pertumbuhan pokok dan mempertingkatkan saiz buah.

Abstract

Tissue-cultured plants of 'Josapine' pineapple were produced from buds obtained from crowns. Their field performance was compared with control plants produced by conventional quartering technique.

Twelve agronomic characters were evaluated viz. fruit weight, plant weight, crown weight, fruit length, fruit diameter, core diameter, slip number, aerial sucker number, ground sucker number, flesh colour, total soluble solids and fruit:plant weight ratio. Tissue-cultured (TC) plants differed significantly from normally propagated plants in all but two of the characters viz. number of aerial suckers and flesh colour. TC plants were less vigorous, had lighter plant mass and produced smaller fruit. The small fruit of TC plants resulted in concomitantly thinner fruit core, lighter crown and an increase in total soluble solids. The population of TC plants also showed higher variability especially in all the fruit characters.

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The most evident somaclonal changes in TC plants were the occurrence of spiny plants (14.7%) and an increase in slip production (up to 7). However, the more worrisome behaviour of TC plants is the poor plant vigour and production of small, uneconomic-sized fruit. Certain agronomic adjustments may be necessary for the management of TC plants to accelerate plant growth and increase fruit size.

Introduction

Pineapple is usually propagated quite easily with suckers, slips and crowns. These vegetative propagules are, to a large extent, produced rather abundantly. However, for some cultivars and under certain situations, shortage of planting materials has been experienced. 'Smooth Cayenne', for example, produces few slips and fewer suckers and the shortage is aggravated when the fruit is sold fresh with the crown.

Several rapid propagation techniques have been developed and may be used to overcome this problem. Lee and Tee (1978) used the plantlet quartering technique that could produce 45 000 plantlets from one mother plant in 18 months. Glennie (1981) used a growth regulator morphactin called Multi-prop (Maintain CF-125) to increase the production of slips in 'Smooth Cayenne' pineapple. For 'Mauritius' pineapple, Heenkenda (1993) mechanically decapitated plants to induce lateral proliferation of suckers. These methods, although increased considerably the rate of planting material production, may still be inadequate when a new variety is developed and very rapid multiplication is needed to quickly introduce it to the industry.

In vitro propagation may be a useful technique under such circumstances. Ting and Chua (1975) successfully developed protocols for in vitro production of the main pineapple cultivars in Malaysia, viz. 'Masmerah', 'Mauritius', 'Singapore Spanish' and 'Smooth Cayenne'. Drew (1980) reported that the in vitro technique has the potential to produce 100 000 plants from a single shoot in 12 months. However, the potential of in vitro propagation of pineapple is clouded by several constraints, notably high costs of production and uncertainty in performance due to somaclonal variation. Variations in leaf colour, phyllotaxy, leaf-spine and wax secretion on leaves were noted in tissue-cultured pineapples (Wakasa 1989). Differential rates of variation were also found when tissues were taken from different organs of the plant. Variations were more pronounced when tissues were cultured from the syncarp compared with those taken from the crown.

MARDI has recently developed a table variety pineapple called 'Josapine', which was released in 1996 (Chan and Lee 1996). Since its release, there has been mounting requests for planting materials, currently produced by the quartering technique. The tissue culture technique seemed a logical choice to ease the backlog in supply. However, the question of somaclonal variation has to be addressed first. This paper examines the field performance of tissue-cultured 'Josapine' pineapple and makes recommendations on the use of the in vitro technique as a way for commercial production of 'Josapine' planting materials.

Materials and methods

Plantlets were initiated from the buds obtained from crowns of 'Josapine' pineapple in MS salts and vitamins (Murashige and Skoog 1962), 0.3% Phytagel (Sigma) and 2.5 mg/litre benzyl amino purine (BAP). Plantlets were rooted in MS salts and vitamins, 0.3% Phytagel and 1 mg/litre naphthalene acetic acid (NAA). Four weeks after root initiation, rooted plantlets were transferred to water-expanded Jiffy bags No. 9 in the greenhouse. After 8 weeks in the

greenhouse, the young plants were transferred to open nursery sand trays where they were kept for another 3 months before field planting.

The experiment was conducted on peat at the MARDI Research Station in Pontian, Johor. The tissue-cultured and control plants of 'Josapine' variety were planted on 21 September 1999 in a randomized complete block design with four replicates. In each plot, 40 plants were grown in two double-row beds of 10 plants in each row. The spacing was 30 cm x 60 cm between plants and 90 cm between beds. The control plants were propagated using the conventional quartering technique of Lee and Tee (1978). All plants in the experiment were selected based on uniform mass of about 200 g each. The number of plants with spiny leaves was counted at field planting. Flower induction ('forcing') was carried out 10 months after field planting with 200 ppm (v/v) ethephon, 2% (w/w) urea and 0.5% (w/w) borax at a rate of 50 mL to a plant.

Data were taken from 10 random plants in each plot. The measurements included the number of ground suckers, aerial suckers and slips, and fresh weights of crown, fruit and plant. Fruit were harvested at the stage when the peel showed half colour. Fruit analyses included diameter and length of the fruit, core diameter, total soluble solids (TSS) and flesh colour. TSS indicating sweetness was measured with a hand refractometer (0-25% Brix). Flesh colour was visually ranked from 1 (white) to 9 (golden-orange).

Results and discussion

Results of the analysis of variance for 12 characters are shown in *Table 1*. Tissue-cultured (TC) plants differed significantly from normally propagated plants in all but two of the characters i.e. number of aerial suckers and flesh colour.

The mean, range and CV of TC vs normally propagated plants are shown in *Table 2*. TC plants grew slower, had smaller plant mass and produced fruit just over half

Table 1. Analysis of variance: mean squares for 12 characters of pineapple grown from two sources of planting materials

Source	df	Fruit weight	Fruit length	Fruit diameter	Core diameter	Slip no.	Aerial sucker no.	Ground sucker no.	Crown weight	Plant weight	Flesh colour	TSS	Fruit:plant ratio
Replicate	3	0.0012 ns	19.55 ns	1.40 ns	0.20 ns	0.04 ns	0.0150 ns	0.60 ns	1 599 ns	0.0668 ns	0.0345 ns	0.4469 ns	0.0003 ns
Planting material	1	0.5392**	1615.96**	215.28**	60.50*	2.88**	0.0050 ns	24.15**	29 161*	1.6790*	0.5512 ns	9.7020**	0.0200**
Error MS	3	0.0084	44.47	3.04	3.42	0.04	0.0017	0.20	1 728	0.0988	0.0579	0.1579	0.0001
Total	7												

* = significantly different at $p < 0.05$
** = significantly different at $p < 0.01$
ns = not significantly different

Table 2. Mean, range and CV of normal vs tissue-cultured 'Josapine' pineapple

	Type of planting material	Mean	Range	CV (%)
Fruit weight (kg)	Normal	1.12a	0.75 – 1.80	19.64
	Tissue-cultured	0.60b	0.40 – 1.05	21.67
Fruit length (mm)	Normal	141.75a	115 – 178	10.17
	Tissue-cultured	113.33b	85 – 150	12.76
Fruit diameter (mm)	Normal	114.05a	105 – 122	4.18
	Tissue-cultured	103.68b	90 – 120	6.43
Core diameter (mm)	Normal	25.23a	21 – 30	8.24
	Tissue-cultured	19.73b	11 – 30	17.89
Slip number	Normal	0.00b	0 – 0	0.00
	Tissue-cultured	1.20a	0 – 7	158.33
Aerial sucker number	Normal	0.35a	0 – 2	165.71
	Tissue-cultured	0.40a	0 – 2	137.50
Ground sucker number	Normal	4.05a	1 – 9	43.95
	Tissue-cultured	0.58b	0 – 3	144.83
Crown weight (g)	Normal	203.50a	100 – 530	48.15
	Tissue-cultured	82.75b	50 – 125	22.73
Plant weight (kg)	Normal	3.49a	1.80 – 5.90	29.51
	Tissue-cultured	2.57b	1.55 – 4.05	20.23
Flesh colour	Normal	6.85a	6 – 8	6.28
	Tissue-cultured	7.38a	7 – 8	6.64
TSS (%)	Normal	15.22b	12.2 – 17.6	8.15
	Tissue-cultured	17.42a	14.0 – 20.4	9.87
Fruit:plant ratio	Normal	0.34a	0.21 – 0.50	20.59
	Tissue-cultured	0.24b	0.16 – 0.30	16.67

Mean values with the same letter are not significantly different at $p = 0.05$ according to the DMRT

the weight of fruit from normally propagated plants. They also showed less efficient partitioning of assimilates to the fruit as reflected by the lower fruit:plant ratio. Perhaps due to the smaller fruit of TC plants, their TSS (17.42%) was higher than that of fruit from normal plants (15.22%). The smaller fruit also resulted in concomitantly thinner fruit cores and smaller crown size. Normal 'Josapine' plants do not produce slips, but a fair number, up to 7, was found in TC plants. Ground sucker production in TC plants was much reduced and this may present a problem in the availability of planting materials for replanting or ratooning.

The population of TC plants generally showed higher variability, especially for fruit characters like dimensions and weight, core size, flesh colour and TSS (*Table 2*). This may be due to somaclonal changes in the TC population. Wakasa (1989) found that somaclonal changes in TC pineapple were considerable, especially when tissues were taken from the syncarp. As a matter of fact, the variation was so great that the TC technique was suggested as a tool to generate variability for exploitation in pineapple selection and improvement.

Somaclonal variation

One of the most obvious changes in this population of TC plants was the occurrence of spines along the leaf margins. Normally propagated 'Josapine' usually have smooth leaf margins with occasional spines at the leaf tips. In this experiment, however, 14.7% of the TC plants have leaves that were completely spiny. Wakasa (1989) also obtained many spiny plants in tissue culture and concluded that the high frequency of

leaf spine variation may be due to spine chimerism already present in donor tissues.

The significant increase in number of slips in TC plants may be a heritable*, bona fide* somaclonal change because normal 'Josapine' plants rarely produce slips. Other than this, the phenotype of TC 'Josapine', plant morphology and fruit characteristics included, were rather identical to normally propagated plants. The differences between the two types of materials i.e. reduced fruit size, crown and core, and reduced number of ground suckers were all physiologically related to the small stature of TC plants at harvest. These differences are, therefore, not considered as somaclonal variation. The limited somaclonal variation was expected because the source materials for culture in this experiment were taken from crown tissues. Wakasa (1989) reported that somaclonal variation from culture of crown buds was only 7% and all involved changes to spiny leaves. Using materials from other organs such as slips and syncarp, somaclones may appear in the range of 98–100%. Drew (1980) suggested that somaclonal variation might not be a problem in TC pineapples because its occurrence can be minimized when sub-cultures were limited to three cycles or less.

A follow-up study is now underway to evaluate the performance of suckers from these TC plants and to determine whether the somaclonal variation can be passed to the next generation. These second-generation TC plants may well revert to normal behaviour and characteristics. This was found to be true in certain grape varieties when TC plants exhibited juvenile characteristics and low yield when first field-planted but these declined or disappeared altogether with aging of the plants (Cancellier and Cossio 1987).

Recommendation and conclusion

The most evident change in TC plants is the occurrence of spiny plants. However, these can be phenotypically identified even at the culture stage and therefore can be eliminated early in the laboratory or later in the nursery. The more worrisome behaviour of TC plants is that first generation materials have poor vigour, grew much slower and when subjected to the same schedule of agronomic practices as normally propagated plants, produced fruit of uneconomic size. It appears that TC planting materials may require a longer gestation period than the currently recommended 10 months before the plants can be subjected to flower induction. This would allow the plants to achieve a larger mass to adequately support an acceptable fruit size.

In general, TC for 'Josapine' may be an acceptable rapid method of propagation because the fruit are phenotypically identical in most aspects to normally propagated ones. However, certain agronomic adjustments may be necessary for the management of first generation TC plants to accelerate the plant growth and increase the fruit size.

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