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Field evaluation of micropropagated and conventionally propagated ginger in subtropical Queensland

M. K. Smith and S. D. Hamill

Queensland Department of Primary Industries, Maroochy Horticultural Research Station, PO Box 5083, SCMC, Nambour, Qld 4560, Australia.

Summary. The growth and performance of micropropagated ginger (*Zingiber officinale* Roscoe) was compared with 'seed'-derived plants in field trials conducted in south-eastern Queensland. In the first generation *ex vitro*, micropropagated plants had significantly (P<0.01) reduced rhizome yield with smaller knobs and more roots. Micropropagated plants had a greater (P<0.01) shoot: root (rhizome) ratio compared with seed-derived plants. Shoots from micropropagated plants were also significantly (P<0.01) smaller with a greater number of shoots per plant. The unusual shoot morphology of the micropropagated plants did not appear to be related to the presence of benzylaminopurine, a plant growth hormone added to the multiplication medium, as plants subcultured for 3 cycles on a hormone-free medium also exhibited similar characteristics. Seed collected from the micropropagated plants and seed-derived plants was harvested and, despite the micropropagated seed being significantly (P<0.01) smaller, by the second generation *ex vitro* there were no significant differences between the treatments. Factors that can improve rhizome size, while reducing production costs, need to be identified before micropropagated plants can be recommended for routine use in the ginger industry as a source of disease and pest-free planting material.

Introduction

In south-eastern Queensland, ginger is grown in an area (about 150 ha) centred on Yandina and 5600 t of rhizomes are processed annually for an estimated value of $A13.5 million.

Ginger is propagated from portions of the rhizome called 'seed'-pieces that have been treated with benomyl (Whiley 1974). Its production is seriously affected by several pests and diseases (Pegg *et al.* 1974). Currently the most serious of these are root-knot nematodes (*Meloidogyne incognita* and *M. javanica*) and Fusarium yellows (*Fusarium oxysporum* f. sp. *zingiberi*). Their effect on crop yields can be greatly exacerbated when infested planting material is used. For example Colbran (1968) found that yield losses of 57% could result when nematode-infested sections of the rhizome, used as seed-pieces, were planted in fumigated soil. *Fusarium* infection of the seed is also serious in that it will continue to destroy rhizome tissues when stored and readily infect plants during all stages of development (Pegg *et al.* 1974).

Micropropagation is an ideal method for mass propagation of pest- and disease-free ginger (Hosoki and Sagawa 1977; De Lange *et al.* 1987; Inden *et al.* 1988); however, little is known about the growth and performance of micropropagated ginger in the field. Recently Bhagyalakshmi *et al.* (1994) compared micropropagated and conventionally propagated ginger in a tropical Indian environment and found lower yields with the micropropagated plants up to 8 months from planting. However, by 10 months, yields from micropropagated plants were not significantly different from conventionally propagated plants. We aimed to compare the growth of micropropagated ginger with plants derived from seed under our subtropical conditions, and investigate possible constraints to the use of micropropagated ginger as a source of uninfected planting material for the Australian ginger industry.

Materials and methods

Plant material

Seed-pieces of ginger (*Zingiber officinale* Roscoe) cv. Queensland were supplied by Buderim Ginger Ltd. This material was used to establish field trials, as well as initiate *in vitro* cultures. Every effort was made to ensure the seed was free from nematodes and *Fusarium*. Average seed weight was about 60 g, unless otherwise stated.

To initiate cultures, seed was surface-sterilised with 1% sodium hypochlorite for 2 min and then stored at ambient temperatures in the laboratory until it began to sprout. Emerging buds (10 mm^3) were removed, surface-sterilised in 3% sodium hypochlorite for 15 min and rinsed 3 times in sterile water. Bleached material was removed and the explant embedded in Murashige and Skoog (1962) basal medium supplemented with 3% sucrose and 2.5 mg/L benzylaminopurine (BAP), and solidified with 0.8% Difco Bacto-agar. Cultures were incubated at 28°C with a 16 h photoperiod. Cool, white fluorescent tubes provided a photon flux density at the culture surface of about 80 µmol quanta/m^2.s.

Shoots proliferated on this medium at the rate of 4–5 per month. The formation of a good root system was also facilitated by this medium and plantlets could be readily deflasked and established in the glasshouse. Plants produced on this medium which contained the hormone, BAP, were referred to as TCH (tissue culture hormone added) plants. An additional treatment involved subculturing plants for 3 successive cycles on hormone-free medium and these plants were referred to as TCF (tissue culture free of added hormone) plants. A good root system was also formed by plantlets growing on this medium. Both tissue culture treatments were subcultured 16 times over 22 months.

Plantlets were deflasked in a sheltered area near the glasshouse. Roots were gently washed free of agar and planted in seedling trays (30 by 50 cm) of steam-pasteurised potting mix. The sand–peat (1 : 1) mixture contained 3.6 kg/m^3 of dolomite and the following nutrients (g/m^3): ammonium sulfate, 544; superphosphate, 184; potassium sulfate, 248; magnesium sulfate, 472; copper sulfate, 7.2; zinc sulfate, 9.6; and iron sulfate, 7.2. Plantlets were watered and enclosed in a plastic tent with 50% shade and grown in a glasshouse with fan-forced heaters and evaporative coolers, with daily temperatures ranging from 20 to 30°C. After 1 week, the plastic was gradually removed until it was completely removed by the end of week 3. Plants were watered as required and the liquid fertiliser Aquasol was applied every 2 weeks at the manufacturer's recommended rate. By the end of week 7, plants were 9 cm tall and ready for establishment in the field.

Experimental procedures and design

Experiment 1. First generation ex vitro. Field trials were conducted on the Australian Golden Ginger Experimental Farm near Kandanga (26°10'S) on a brown, clay–loam soil. Previous experience with micropropagated ginger (A. W. Whiley pers. comm.) established the need to grow plants under shade to reduce the risk of plant loss during the hot, dry conditions that are frequently experienced in late spring and early summer. Two 15 by 5 m shadehouses (50% shade) were constructed on an area previously used for ginger production. Ethylene dibromide (EDB 193%) had been injected into the site with tined fumigation equipment at a rate of 50 L/ha before the shadehouses were erected and used for the establishment of micropropagated ginger (Smith and Drew 1990). Only micropropagated ginger had been grown in these shadehouses before commencement of the experiments.

Before preparing beds for planting, the area was rotary hoed and all sections of ginger rhizome from previous trials were completely removed. Mill mud (an organic soil amendment consisting of residual material from the processing of sugar cane) was applied at a rate of 125 t/ha and incorporated to a depth of 10 cm with a rotary hoe. Two weeks before planting, the soil was formed into beds about 150 mm high by 1.5 m wide, with 2 beds in each shadehouse. A few days before planting, emerging weeds were sprayed with Spray Seed (paraquat/diquat) at a rate of 3.5 L/ha. Subsequent weeds were removed by hand. Aquasol was applied at the manufacturer's recommended rate 1 week after planting. Subsequently, a split application of granulated superphosphate (9.6% phosphorus) and Crop King Q7(K) (10.9% nitrogen, 2% phosphorus, 21.1% potassium) was applied at 1000 and 600 kg/ha, respectively, with 20% as a basal application, followed by 40% in mid December and 40% in early February. Nemacur 10G (10% fenamiphos) was applied in mid December at 110 kg/ha. Overhead sprinklers, installed over each bed, provided irrigation as required.

Micropropagated plants and seed were planted on 20 October 1992 in a randomised block design with 8 replicates and 3 treatments (seed, TCH, TCF). Each block consisted of a 3-row bed, about 5 m long, with 30 cm between plants along the row and 40 cm between rows. Micropropagated plants and seed were planted by hand, with the seed planted to a depth of about 10 cm. All micropropagated plants survived transplanting; however, 12.5% of seed failed to grow. The middle row of each treatment was used as the sampling unit with a single plant taken from the centre for the early harvest, and 3 plants taken for the seed harvest. These 3 plants were also used to measure shoot height (tallest) and number at different stages during plant growth. Data were analysed by ANOVA.

The first harvest (early harvest) took place on 5 April 1993 when the flower heads had emerged. This corresponded with a period of maximum recovery of 'choice' grade ginger. Choice grade ginger (used for confectionery) is when 35–45% by weight of the rhizome is free from commercial fibre ('fibre-free') (Whiley 1979). The final seed harvest took place on 1 September 1993, 5 weeks before the seed was used for the next experiment. In addition to sampling plants, all border plants from each treatment were also harvested. Following measurements, rhizomes were stored in hessian bags in a dry, well-ventilated room.

Experiment 2. Second generation ex vitro. Experiment 2 was planted in the field in an area set aside for the commercial production of seed ginger. To ensure the site was reasonably free from nematodes, it had been left fallow for 1 season and, prior to the ginger being planted, was cropped with maize, incorporated to facilitate the breakdown of organic material. Soil preparation and agronomic practices were essentially as described for experiment 1. However, as the experiment was no longer confined to a shadehouse, broad-acre farming practices could be adopted with fertiliser,

nematicide and herbicide applications. Therefore, while the rates remained the same, the implementation was different. Spray Seed was used as a pre-emergence herbicide at 3.5 L/ha, and for spot-spraying weeds after crop establishment. Additional weed control was achieved by applying 4.5 kg/ha of Diuron (without surfactant) as shoots emerged but before leaves started to expand. Overhead sprinklers provided irrigation and were essential to protect the crop from sunburn during late spring–early summer.

Rhizomes from experiment 1 were cut into seed-pieces of 2 sizes: small, 35–45 g; and large, 55–65 g. Seed was treated for 10 min with 1 g/L Benlate (0.5 g/L benomyl) and air-dried before being stored for 5 weeks in a cool, dry place until planting on 6 October 1993. The 6 treatments consisted of 3 sources of planting material (seed, TCH, TCF) at each of 2 sizes (small and large). The design was a randomised block with 4 replicates. Insufficient planting material resulted in the small TCH and small seed treatments only being applied to 3 blocks. Each block consisted of a 3-row bed, 1.8 m wide by 20–25 m long. To ensure uniform spacing, seed-pieces were planted by hand to a depth of 10 cm with 30 cm between plants along the row and about 40 cm between rows. Only 2.5% of seed derived from micropropagated plants failed to grow compared with 5% of the conventional material. The middle row of each treatment was used as the sampling unit with 3–5 plants taken from the centre for early harvest (23 March 1994), and 5–10 plants taken for seed harvest (24 August 1994). Data were analysed by ANOVA.

Measurements

After planting in the shadehouses, plants were inspected monthly and the number of shoots and height (cm) of the tallest shoot were taken for each plant. At early harvest, plants were pulled from the ground and hosed to remove soil before the following measurements were taken: number of shoots, length of shoot (cm), total shoot fresh weight (g_{fw}), rhizome fresh weight (g_{fw}), root fresh weight (g_{fw}) and number of rhizome knobs. From the rhizome weight and number of knobs, mean knob size was calculated. This feature is important as large knobs are favoured during factory processing and for the sale of fresh rhizomes. The recovery of choice grade ginger is also an important consideration for product quality (Leverington 1969) and was determined using the standard commercial blunt knife technique (Whiley 1980). A subsample of 5 shoots was randomly selected from each plant, and the number of leaves and leaf area (cm^2) per shoot determined. From these measurements total leaf area per plant could be estimated.

At seed harvest, shoots had senesced with the onset of cooler weather. Plants were removed from the ground and hosed to remove soil before the following measurements were taken: rhizome fresh weight (g_{fw}), root yield (g_{fw}) and number of knobs.

Results

Experiment 1. First generation ex vitro

There were significant differences between micropropagated ginger and plants derived from seed. The first generation of plants out of culture were smaller ($P<0.01$) than seed-derived ginger at all stages of growth and produced a significantly ($P<0.01$) greater number of shoots (Fig. 1*a* and *b*). This was irrespective of whether

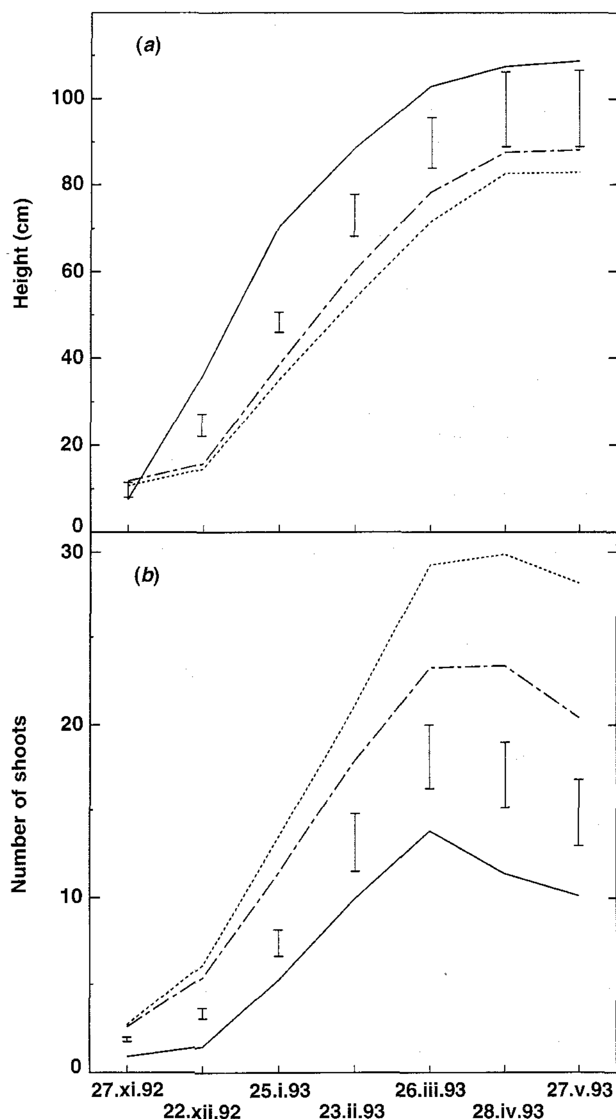

Figure 1. Comparison of (*a*) shoot height and (*b*) number of shoots per plant with 3 sources of ginger planting material: seed (——); micropropagated plantlets growing on hormone-free medium (TCF, ...); micropropagated plantlets growing on benzylaminopurine-containing medium (TCH, – – –). Values are the means of 24 replicates. Vertical bars indicate l.s.d. values at $P = 0.01$.

Table 1. Shoot characteristics of first generation *ex vitro* micropropagated and seed-derived ginger plants at early harvest

Plants were derived from seed or micropropagated on either hormone-free medium (TCF) or benzylaminopurine-containing medium (TCH)
Values are means of 8 replicates
Means within each row followed by the same letter are not significantly different at the *P* values indicated

Characteristic	Seed	TCH	TCF	l.s.d. ($P = 0.01$)	l.s.d. ($P = 0.05$)
Total shoot mass (g_{fw})	805.3a	791.8a	514.9a	n.s.	n.s.
No. of shoots	13.1a	30.0b	31.8b	12.1	
Mean shoot mass (g_{fw})	61.1a	27.9b	16.1c	11.4	
Shoot length (tallest) (cm)	111.3a	84.9b	70.6c	17.4	12.5
Mean shoot length (cm)	85.5a	62.7b	50.5c	13.7	9.9
No. of leaves per shoot	17.4a	15.4ab	14.3b	2.9	
Mean leaf area (cm^2)	50.6a	34.9b	26.6c	8.6	6.2
Leaf area per shoot (cm^2)	900.9a	536.7b	382.7c	171.6	123.1
Plant leaf area (cm^2)	13 333a	15 412a	12 459a	n.s.	n.s.

plants were multiplied on BAP-containing medium (TCH) or whether they had been subcultured for 3 cycles on hormone-free medium (TCF).

Early harvest

At harvest, seed-derived plants had fewer shoots that were taller and heavier ($P<0.01$), and with greater leaf number and individual leaf area, than those of micropropagated plants (Table 1).

Comparisons with shoots from the micropropagated treatments showed that TCH plants had larger shoots with greater leaf area than TCF plants ($P<0.01$). However, when total shoot mass and leaf area were compared there were no significant differences between the 3 treatments (Table 1). Micropropagated plants produced fewer inflorescences (21% TCF; 4% TCH plants flowering) than those derived from seed (91% flowering) (data not shown).

The rhizome from seed-derived ginger was significantly ($P<0.01$) heavier with larger knobs and less root mass than rhizomes from micropropagated ginger (Table 2). There were also proportionally less roots (expressed as a percentage of total rhizome mass) on seed-derived plants ($P<0.01$). TCH plants grew taller, had larger leaves with greater leaf area per shoot and produced more rhizome than TCF plants, but in most other aspects were similar (Tables 1 and 2). Partitioning of biomass between shoots and rhizomes favoured the rhizome in seed-derived plants (shoot : rhizome ratio <1.0), and shoots in micropropagated plants (shoot : rhizome ratio >1.0). Shoot : rhizome ratios were significantly different ($P<0.05$) between treatments with TCF plants more biased to shoot growth than TCH plants (Table 2).

Seed harvest

Differences in rhizome characteristics determined at early harvest were still apparent between treatments 5 months later at seed harvest. With respect to rhizome

Table 2. Rhizome characteristics of first generation *ex vitro* micropropagated and seed-derived ginger plants at early harvest

Plants were derived from seed or micropropagated on either hormone-free medium (TCF) or benzylaminopurine-containing medium (TCH)
Values are means of 8 replicates
Means within each row followed by the same letter are not significantly different at the *P* values indicated

Characteristic	Seed	TCH	TCF	l.s.d. ($P = 0.01$)	l.s.d. ($P = 0.05$)
Rhizome mass (g_{fw})	920.8a	525.4b	202.0c	442.2	318.6
Root mass (g_{fw})	17.9a	65.5b	79.3b	38.4	
Percentage of roots	2.0a	13.7b	30.2c	14.9	10.7
No. of knobs	85.5a	105.8a	72.0a	n.s.	n.s.
Mean knob mass (g_{fw})	11.1a	4.9b	2.9b	2.8	
Total mass (g_{fw})	991.6a	590.9b	281.3b	450.5	324.6
Shoot : root (rhizome) ratio	0.80a	1.42b	1.87c	0.59	0.42

Table 3. Rhizome characteristics of first generation *ex vitro* micropropagated and seed-derived ginger plants at seed harvest

Plants were derived from seed or micropropagated on either hormone-free medium (TCF) or benzylaminopurine-containing medium (TCH)
Values are means of 24 replicates
Means within each row followed by the same letter are not significantly different at the P values indicated

Characteristic	Seed	TCH	TCF	l.s.d. ($P = 0.01$)	l.s.d. ($P = 0.05$)
Rhizome mass (g_{fw})	958.7a	462.9b	338.2b	228.7	
Root mass (g_{fw})	30.8a	49.3ab	87.4b	55.5	
Percentage of roots	3.8a	9.8b	20.0c	7.3	5.3
No. of knobs	87.1a	87.2a	94.4a	n.s.	n.s.
Mean knob mass (g_{fw})	10.9a	5.3b	3.6c	1.76	1.27
Total mass (g_{fw})	989.5a	512.2b	425.5b	232.6	

weight, seed-derived plants outyielded TCH and TCF plants by 207 and 283%, respectively ($P<0.01$) (Table 3). There was no significant difference in the number of knobs per rhizome between treatments but plants derived from seed had significantly larger knobs ($P<0.05$) than micropropagated plants. However, while there was no significant difference between knob size of TCH and TCF plants at early harvest, by seed harvest knobs of TCH plants were significantly ($P<0.05$) larger (Tables 2 and 3).

Seed prepared from rhizomes harvested in experiment 1, also showed some differences. For example seed collected from seed-derived plants was significantly ($P<0.01$) heavier with fewer, larger knobs compared with micropropagated plants (Table 4).

Experiment 2. Second generation ex vitro

When preparing seed from the first generation of *ex vitro* plants for experiment 2, 63% of TCH and 78% of TCF rhizomes were discarded due to small, poorly developed knobs unsuitable for seed use, or low-level *Fusarium* infection (<10%). In contrast, with material from seed-derived plants, 55% of rhizomes were discarded mainly due to *Fusarium* rhizome rot and the rigorous selection of only the best, uninfested seed.

Second generation *ex vitro* plants originally derived from micropropagation, grew as well or better than plants which had always been propagated from seed. TCH plants appeared more vigorous and by early harvest had significantly ($P<0.05$) more shoots with a greater total shoot mass than seed-derived plants (Table 5). However, there were no significant differences in rhizome yield or other rhizome characteristics between treatments although TCH plants produced more roots than plants grown from seed (Table 6). At seed harvest 5 months later, there were no differences in rhizome or root characteristics between treatments irrespective of seed size or whether the plants had been micropropagated or always grown from seed (Table 7). Fusarium yellows was less pronounced with 4% infection of the rhizomes originally derived from micropropagation compared with 7% of the rhizomes from seed-derived plants (data not shown).

At both early harvest and seed harvest, there were no significant differences in any parameter measured between small- and large-seed classes. The smaller seed pieces (35–45 g) planted at a density of 67 000 plants/ha,

Table 4. Seed characteristics of first generation *ex vitro* micropropagated and seed-derived ginger plants at planting

Rhizomes were harvested from first generation *ex vitro* micropropagated plants grown on either hormone-free medium (TCF) or benzylaminopurine-containing medium (TCH) or from seed-derived plants from experiment 1
Seed from these rhizomes was graded small (35–45 g) or large (55–65 g)
Values are means of a subsample of 25 seeds from each treatment (seed, TCF, TCH x small, large) after storage for 5 weeks
Means within each row followed by the same letter are not significantly different at $P = 0.01$

Characteristic	Seed	TCH	TCF	l.s.d. ($P = 0.01$)	Seed size (g) Small	Seed size (g) Large	l.s.d ($P = 0.01$)
Seed mass (g_{fw})	46.4a	42.6b	42.1b	3.2	35.8a	51.6b	2.7
No. of knobs	5.58a	6.88b	7.28b	0.91	6.47a	6.69a	n.s.
Mean knob mass (g_{fw})	8.64a	6.48b	6.38b	1.07	6.18a	8.15b	0.88

Table 5. Shoot characteristics of second generation *ex vitro* micropropagated and seed-derived ginger plants at early harvest

Plants were derived from first generation *ex vitro* micropropagated plants grown on either hormone-free medium (TCF) or benzylaminopurine-containing medium (TCH) or from seed-derived plants from experiment 1

Values are means of 12–20 replicates

Means within each row followed by the same letter are not significantly different at $P = 0.05$

Characteristic	Seed	TCH	TCF	l.s.d. ($P = 0.05$)
Total shoot mass (g_{fw})	823.1a	1183.3b	927.6ab	304.4
No. of shoots	20.5a	29.3b	24.6ab	7.5
Mean shoot mass (g_{fw})	39.8a	41.0a	37.9a	n.s.
Shoot length (tallest) (cm)	78.7a	84.9a	83.0a	n.s.

gave the same yield as larger seed pieces (55–65 g) planted at the same density (data not shown).

Discussion

Micropropagated ginger is an excellent source of disease- and nematode-free planting material (Hosoki and Sagawa 1977; De Lange *et al.* 1987; Inden *et al.* 1988). However, our study shows that growth and yield of rhizome is inferior to ginger propagated from conventional seed sources. This difference only occurs in the first generation of plants from tissue culture. By the second generation, plants originally derived from micropropagation were indistinguishable from plants that had always been propagated from seed. Even though seed derived from first generation *ex vitro* plants was generally smaller in mass and knob size.

Table 6. Rhizome characteristics of second generation *ex vitro* micropropagated and seed-derived ginger plants at early harvest

Plants were derived from first generation *ex vitro* micropropagated plants grown on either hormone-free medium (TCF) or benzylaminopurine-containing medium (TCH) or from seed-derived plants from experiment 1

Values are means of 12–20 replicates

Means within each row followed by the same letter are not significantly different at $P = 0.01$

Characteristic	Seed	TCH	TCF	l.s.d. ($P = 0.01$)
Rhizome mass (g_{fw})	702.3a	950.9a	723.0a	n.s.
Choice grade mass (g_{fw})	285.6a	358.3a	278.3a	n.s.
Percentage of choice grade	41.1a	37.9a	39.0a	n.s.
Root mass (g_{fw})	9.9a	25.5b	15.8ab	14.5
Percentage of roots	1.36a	2.54a	3.71a	n.s.
No. of knobs	74.0a	99.9a	78.3a	n.s.
Mean knob mass (g_{fw})	9.55a	9.56a	8.86a	n.s.
Total mass (g_{fw})	712.2a	975.9a	738.8a	n.s.
Shoot : root (rhizome) ratio	1.13a	1.20a	1.52a	n.s.

Table 7. Rhizome characteristics of second generation *ex vitro* micropropagated and seed-derived ginger plants at seed harvest

Plants were derived from first generation *ex vitro* micropropagated plants grown on either hormone-free medium (TCF) or benzylaminopurine-containing medium (TCH) or from seed-derived plants from experiment 1

Values are means of 20–40 replicates

There were no significant differences between treatments

Characteristic	Seed	TCH	TCF
Rhizome mass (g_{fw})	1167.0	1188.0	1391.2
Root mass (g_{fw})	19.5	26.8	21.5
Percentage of roots	1.75	2.20	1.66
No. of knobs	107.8	115.8	130.1
Mean knob mass (g_{fw})	10.8	10.3	10.6
Total mass (g_{fw})	1236.9	1262.8	1466.9

We have identified a number of constraints for the use of micropropagated ginger as a source of uninfected seed. First, micropropagated plants are grown in enclosed containers under closely controlled laboratory conditions. During deflasking they are prone to desiccation, and overwetting of leaves can cause soft rots to develop. Both of these problems can be related to poor cuticular development on leaves grown *in vitro* and, therefore, care is needed during deflasking and acclimatisation in the glasshouse to achieve good establishment. Ginger is also particularly prone to sunburn (Whiley 1974); therefore, growth of micropropagated ginger under shade should be taken as a precaution to prevent plant loss.

Because of the need of special facilities and greater levels of management to ensure survival and growth, production of seed from micropropagated plants will be more expensive than seed obtained by conventional practices.

Second, during the first generation *ex vitro*, rhizomes produced from micropropagated plants were smaller than from seed-derived sources and there was more wastage due to a greater mass of roots and small, poorly developed knobs that cannot be used as seed. This also adds to the cost of seed obtained from micropropagated plants.

Third, Fusarium yellows is widespread in the industry and once introduced the organism can remain in the soil for many years (Pegg *et al.* 1974). In our study, a small percentage of rhizome grown from micropropagated plants was infected with Fusarium yellows. This highlights the persistence of *Fusarium oxysporum* in soil since the experimental site for the first generation crop was replant ground and plants were disease-free from tissue culture. However, despite rigorous selection of conventional seed, rhizomes produced from this source of

planting material had a much higher level of Fusarium yellows at harvest indicating its presence either in original seed-pieces or the increased opportunity for invasion through cut surfaces which occur during seed preparation (Whiley 1974).

Hence, for seed production, a nursery area should be chosen which has never produced ginger. Stringent quarantine practices are required to prevent introduction of Fusarium yellows and nematodes. Even though nematicides or various organic amendments can be used to control root-knot nematode (Stirling 1989), a good practice for seed production would be to only plant disease- and nematode-free material from tissue culture in uninfested ground.

Micropropagation has already found an important niche in the Australian ginger industry by allowing the rapid multiplication of promising new cultivars which are then propagated by more conventional practices (Smith and Drew 1990). However, if micropropagation is to be used more routinely for the production of uninfected seed, factors must be identified that can improve rhizome size, reduce wastage and, therefore, improve seed recovery.

In a previous study, M. K. Smith and S. D. Hamill (unpublished data) found a 2.6-fold decrease in rhizome yield with micropropagated plants compared with seed-derived plants, with a corresponding increase in number of shoots, even though total fresh weight of shoots and total leaf area were essentially the same. It was thought that the cytokinin, BAP, which is known to promote shoot initiation *in vitro* (George and Sherrington 1984), gave a carry-over effect promoting excessive vegetative growth of micropropagated plants established in the field. The experiments reported here indicate that BAP added to the culture medium was probably not responsible for these effects. Plantlets subcultured on hormone-free medium behaved similarly, although plants grown on medium containing BAP (TCH) consistently outperformed those grown on hormone-free medium (Tables 1–3). There was also some indication that at early harvest, seed recovered from TCH plants were a better source of planting material compared with TCF plants (Tables 5–6). However, even these differences were not obvious by seed harvest (Table 7).

There are other reasons that may account for smaller rhizomes in the micropropagated plants during their first generation *ex vitro*. First, micropropagated plants have no seed reserve. Plantlets at deflasking are 4–5 cm tall and weigh less than 1 g whereas the seed has no shoots at planting and weighs about 60 g. Contrasts between the 2 forms of planting material could not be more striking. Whiley (1980) and Okwuowulu (1988) showed that seed is an important source of assimilate for the developing plant and the amount of seed reserve, and as a consequence the growth of the first order shoot, have a large effect on knob size and final yield. This difference probably accounts for the major yield differences between micropropagated and seed-derived plants. Bhagyalakshmi *et al.* (1994), with an Indian ginger variety, also found significantly lower yields with micropropagated ginger harvested at 8 months compared with seed-derived ginger, and also attributed their difference to micropropagated plants lacking a rhizome (seed reserves) when planted. Transplant shock was also given as a reason for the lower yield potential and our own data suggests that some stress may act in the first month after planting that slows growth (Fig. 1*a*). Whether slower growth results from water stress or sunburn remains to be investigated. Bhagyalakshmi *et al.* (1994) found their yields were more comparable at 10 months as the plants continued to grow rapidly under the more tropical conditions at Mysore, India (12°18'N).

Second, micropropagated plants are characterised by plants with many small shoots, and the shoot : root (rhizome) ratio is higher than for seed-derived plants. Our hypothesis that the presence of BAP in the culture medium may have contributed to more vegetative growth in the field was not supported by our data. Another explanation may involve rejuvenation of the material following extended periods of culture. In strawberries, for example, rejuvenation was associated with more vigorous vegetative growth, especially stolon production, and these juvenile characters became less apparent as the plants matured (Huxley and Cartwright 1994). The cause of tissue-culture-induced rejuvenation is unknown; however, if various cytokinin-overproducing states exist in *in vitro* plants, the effect on the *ex vitro* phenotype can be rejuvenating (Swartz 1991). Three subcultures on a hormone-free medium may, therefore, have been insufficient to reverse a possibly habituated, cytokinin-autonomous, culture state. The fact that seed obtained from micropropagated plants produces plants of normal morphology strongly supports the plants not being genetic variants or offtypes that have arisen during micropropagation.

Third, photoperiod is also known to effect rhizome development in ginger. Adaniya *et al.* (1989) suggest that ginger is a quantitative short-day plant and that long days tend to enhance vegetative growth while rhizome swelling is promoted by short days. Because our plants were cultured under 16 h daylength we can speculate that the plants did not receive the induction necessary to promote rhizome development.

Flowering was also affected in the micropropagated ginger and this may also indicate a photoperiod response, although Adaniya *et al.* (1989) were unable to show a clear response in 'time to flowering' or 'flower number' to daylength in 3 Japanese ginger cultivars. Okwuowulu (1988) studied the effect of seed-piece weight on flowering in 2 Nigerian cultivars of ginger and

found that the number of inflorescences per plant increased in both cultivars as the seed-piece weight was increased from 5 to 40 g. Therefore seed reserve may not only have an impact on rhizome development, but may also affect flowering.

Conclusion

A full cost-benefit analysis of the use of micropropagated ginger is needed before it can be recommended as a source of uninfected planting material for the ginger industry. We have demonstrated that growth and performance of plants derived from culture is as good as plants propagated by seed after the second generation *ex vitro*. However, there are constraints to production of the first generation of micropropagated plants such as the need for laboratories to produce plantlets and special facilities for deflasking and producing the first crop of seed, as well as the greater level of management required to ensure their survival and growth. We were also able to demonstrate constraints due to lower rhizome yields, the higher level of wastage due to poor rhizome characteristics and the need to ensure the nursery area is free from nematodes and Fusarium yellows. In the meantime, conditions need to be identified for improving rhizome size and recovery of disease and pest-free seed, while reducing production costs. By gaining a better understanding of the factors influencing rhizome development, progress can be made in the provision of a micropropagated plant better able to meet the needs of the Australian ginger industry.

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