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Use of tissue culture for ginger propagation and improvement

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The use of tissue culture for ginger propagation and improvement

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THE USE OF TISSUE CULTURE FOR GINGER PROPAGATION AND IMPROVEMENT

HRDC PROJECT NO. VG104

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1. Final Report Summary

a) Industry summary:

Fusarium and nematode infection of ginger planting material are the two biggest threats to clean, sustainable ginger production in Australia. Tissue culture has the potential to produce disease and pest-free planting material, however the growth and performance of micropropagated ginger under Australian conditions has never been documented. This study was undertaken to determine the feasibility of using micropropagated ginger as a source of 'clean' planting material for the Australian ginger industry.

Micropropagated ginger requires special handling during deflasking and provision of a humid, shaded environment is critical to successfully establish ginger in pots. The use of shadehouses in the field was also found to be important for the growth of micropropagated ginger, as they are exceptionally prone to sunburn and desiccation. Unfortunately the yield of the first generation of ginger out of culture was much poorer than ginger obtained from 'seed'-pieces. Micropropagated ginger was characterised by smaller rhizomes with many, small knobs and a greater percentage of roots. However, by the second generation, 'seed' obtained from the micropropagated plants was equal in performance to 'seed' obtained from conventionally propagated ginger.

A full cost-benefit analysis of the use of micropropagated ginger is needed before it can be recommended as a source of 'clean' 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. These include the need for laboratories to produce plantlets, the need for special facilities to deflask and produce the first crop of 'seed', the greater level of management required to ensure their survival and growth, the 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 that can improve 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 ginger industry.

b) Technical 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* the micropropagated plants produced significantly (P<0.01) smaller rhizomes with many small knobs and excessive roots. The micropropagated plants were also more vegetative, with the fresh weight ratio of shoots: rhizome (roots) significantly (P<0.01) greater with the micropropagated plants as compared to the 'seed'-derived plants. The shoots from the micropropagated plants were also significantly (P<0.01) smaller with a greater number of shoots per plant. The excessive vegetative nature of the micropropagated plants did not appear to be as a consequence of the cytokinin, benzylaminopurine, which was included in 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 mircropropagated 'seed' being significantly (P<0.01) smaller, by the second generation *ex vitro* there were no significant differences between the treatments.

Micropropagated ginger offers clear advantages as a source of disease and pest-free planting material. However there are currently constraints to the production of 'clean-seed', by this method. These constraints include the need for laboratories to produce the plantlets, the need for special facilities to deflask and produce the first crop of 'seed', the greater level of management required to ensure their survival and growth, the lower rhizome yields in the first generation, the higher level of wastage due to poor rhizome characteristics in the first generation and the need to ensure the nursery area is free from nematodes and Fusarium. 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 'clean' planting material.

c) Publication:

Draft paper (see attached) has been submitted to 'Australian Journal of Experimental Agriculture'.

Field evaluation of micropropagated ginger in subtropical Queensland

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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 to 'seed'-derived plants and 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 in the multiplication medium, as plants subcultured for three 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

Ginger is grown in an area centred on Yandina in south-eastern Queensland. From a total area of approximately 150 ha, 5600 t of rhizomes are processed annually for an estimated value of $13.5 million.

Ginger production is seriously affected by a number of 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 instance Colbran (1968) found that yield losses of 57% could result when nematode infested sections of the rhizome, which are 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 will readily infect plants during all stages of development (Pegg *et al.* 1974).

Micropropagation is an ideal method for the mass propagation of pest and disease-free ginger (Hosoki and Sagawa 1977, De Lange *et al.* 1987, Inden *et al.* 1988), however published information regarding the growth and performance of micropropagated ginger in the field was lacking when these experiments commenced in 1992. The present study was undertaken to compare the growth of micropropagated ginger with plants derived from 'seed' and to investigate possible constraints to the use of micropropagated ginger as a source of 'clean' planting material for the ginger industry.

Materials and methods

Plant material

Ginger is propagated from portions of the rhizome called 'seed'-pieces that have been treated with benomyl (Whiley 1974). 'Seed'-pieces of ginger (*Zingiber officinale* Roscoe) cv. Queensland were supplied by Buderim Ginger Ltd. This material was used to establish the field trials, as well as to initiate *in vitro* cultures. Every effort was made to ensure the 'seed' was free of nematodes and Fusarium. The average 'seed' weight was approximately 60 g, unless otherwise stated. To initiate cultures, 'seed' was surface sterilised with 1% sodium hypochlorite for two minutes and then stored at ambient temperatures in the laboratory until it began to sprout. Emerging buds, 10 mm^3, were removed and surface sterilised in 3% sodium hypochlorite for 15 minutes and rinsed three times in sterile water. Bleached material was pared and the explant embedded on Murashige and Skoog (1962) basal medium supplemented with 3% sucrose and 2.5 mg l^{-1} benzylaminopurine (BAP) and solidified with 0.8% Difco Bacto-agar. The 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 ca.80 µmol quanta m^{-2} s^{-1}. Multiplication rates of 4-5 per month were obtained with this medium. 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 containing a hormone, BAP, were referred to as TCH. An additional treatment involved subculturing plants for 3 successive cycles on a hormone-free medium and these plants were referred to as TCF. A good root system was also formed by plantlets growing on this medium.

Plantlets were deflasked in a sheltered area near the glasshouse. Roots were gently washed free of agar and planted in speedling trays (30 x 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). The 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 20-30°C. After 1 week, the plastic was gradually removed until it was completely removed by the end of the third week. The plants were watered as required and the liquid fertilizer Aquasol ® was applied fortnightly at the manufacturer's recommended rate. By the end of the seventh week 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 Property near Kandanga (26° 10'S) on a brown, clay-loam soil. Previous experience with micropropagated ginger (Whiley, pers. comm.) established the need to grow the plants under shade to reduce the risk of plant loss during hot, dry conditions that are frequently experienced in late spring and early summer. Two 15 m x 5 m shadehouses (50% shade) were constructed on an area that had previously been 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^{-1} 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 prior to the commencement of the experiments reported in this paper.

Before bedding up for planting, the area was rotary hoed and all sections of ginger rhizome from previous trials 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^{-1} and incorporated to a depth of 10 cm with a rotary hoe. Two weeks before planting the soil was formed into beds approximately 150 mm high x 1.5 m wide, with two beds in each shadehouse. A few days prior to planting, emerging weeds were sprayed with Spray.Seed (paraquat/diquat) at a rate of 3.5 l ha^{-1}. Subsequently weeds were removed by hand. The liquid fertilizer Aquasol ® was applied at the manufacturer's recommended rate one week after planting. Subsequently a split application of granulated superphosphate (9.6%P) and Crop King Q7(K) (10.9% N, 2% P, 21.1% K) was applied at a rate of 1000 kg ha^{-1} and 600 kg ha^{-1}, 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 a rate of 110 kg ha^{-1}. 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 eight replicates used to assess the effects of three treatments ('seed', TCH and TCF). Each block consisted of a three-row bed, approximately 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' being planted to a depth of about 10 cm. All micropropagated plants survived the transplanting, however, 12.5% of the 'seed' failed to grow. The middle row of each treatment was used as the sampling unit with a single datum plant taken from the centre for the early-harvest and three datum plants taken for the 'seed'-harvest. The three datum plants were also used to measure shoot height (tallest) and number at different stages during the growth of the plants. Data were analysed by ANOVA.

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

Experiment 2 - second generation ex vitro

The second experiment 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 one season and, prior to the ginger being planted, was cropped with maize which was incorporated to facilitate the breakdown of organic material. Soil preparation and agronomic practices were essentially those used in the previous experiment, however no longer being confined to a shadehouse meant 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 (paraquat/diquat) was used as a pre-emergence herbicide at a rate of 3.5 l ha^{-1} and also for spot spraying weeds after the crop was established. Additional weed control was achieved by the application of 4.5 kg ha^{-1} of Diuron (without surfactant) as the shoots emerged but before the 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 two size classes; small (35-45 g) and large (55-65 g). The 'seed' was treated for 10 minutes with 1 g l^{-1} Benlate® (0.5 g l^{-1} benomyl) and air-dried before being stored in a cool, dry place until planting on 6 October 1993. The six treatments consisted of three sources of planting material ('seed', TCH and TCF) at each of two sizes (small and large). The design was a randomised block with four replicates. Insufficient planting material resulted in the small TCH and small 'seed' treatments only being applied to three blocks. Each block consisted of a three-row bed 1.8 m wide and 20-25 m long. To ensure uniform spacing, the 'seed'-pieces were planted by hand to a depth of 10 cm with 30 cm between plants along the row and approximately 40 cm between rows. Only 2.5% of the 'seed' derived from micropropagated plants failed to grow compared to 5% of the conventional material. The middle row of each treatment was used as the sampling unit with between 3-5 datum plants taken from the centre for the early-harvest (23 March 1994) and between 5-10 datum plants taken for the '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 the 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. During commercial ginger production plants are mechanically pulled at early harvest, and although our method gives a realistic measurement of root recovery at early harvest, it can not give a completely accurate measurement of root mass because during pulling roots are broken and remain in the ground. 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 sub-sample of 5 shoots was randomly selected from each plant and the number or leaves and leaf area (cm^2) shoot $^{-1}$ determined. From these measurements total leaf area per plant could be estimated.

At 'seed' harvest shoots had senesced with the onset of cooler weather and plants were dug 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

From the beginning 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 its growth and produced a significantly ($P<0.01$) greater number of shoots (Figure 1). This was irrespective of whether the plants were multiplied on a BAP medium (TCH) or whether they had been subcultured for 3 cycles on a hormone-free medium (TCF).

Early Harvest

At harvest 'seed'-derived plants had fewer shoots which were significantly ($P<0.01$) taller and heavier, and with greater leaf number and area than those of micropropagated plants (Table 1).
Comparisons between shoots from micropropagated treatments showed that TCH plants had larger shoots with greater leaf area than TCF plants ($P<0.01$). However, when total plant mass and leaf area were compared there were no significant differences between any of the treatments (Table 1). Micropropagated plants produced fewer inflorescences (21% of TCF; 4% of TCH plants flowering) than those derived from 'seed' (91% flowering).

The rhizome from 'seed'-derived ginger was significantly ($P<0.01$) heavier with larger knobs and less root mass than rhizomes from micropropagated ginger treatments (Table 2). There were also proportionally less roots (expressed as a percentage of total rhizome mass) on 'seed'-derived plants compared with ginger from micropropagated treatments ($P<0.01$). TCH plants grew taller, had larger leaves with greater leaf area shoot $^{-1}$ and produced more rhizome than TCF plants, but in most other aspects were similar (Tables 1 and 2). Partitioning of biomass between shoots and rhizome 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 five months later when the 'seed' harvest was made. With respect to rhizome weight, 'seed'-derived plants out-yielded TCH and TCF plants by 207 and 283%, respectively ($P<0.01$) (Table 3). There were no significant difference in the number of knobs rhizome^{-1} 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 than TCF plants (Table 2 and 3).

The 'seed' prepared from the rhizomes harvested in Experiment 1, also showed some differences. For instance the 'seed' collected from 'seed'-derived plants was significantly ($P<0.01$) heavier with fewer, larger knobs as compared to the 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, 'clean' 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). Five months later at 'seed'-harvest there were no differences in rhizome or root characteristics between treatments (Table 7).

A final observation to make regards the effect of the two seed size classes on rhizome yield. At both early-harvest and 'seed'-harvest there were no significant differences in any parameter measured between small and large seed. In other words, the smaller seed pieces (35-45 g) planted at a density of 67,000 plants ha^{-1}, gave the same yield as larger seed pieces (55-65 g) planted at the same density.

Discussion

Micropropagated ginger is an excellent source of disease and nematode-free planting material (Hosaki and Sagawa 1977, De Lange *et al.* 1987, Inden *et al.* 1988), however, our study has shown that growth and yield of rhizome is inferior to that of ginger that has been 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'. This is despite the fact that 'seed' derived from first generation *ex vitro* plants was generally smaller in mass and knob size.

We have identified a number of constraints for the use of micropropagated ginger as a source of 'clean seed'. These can be summarised as follows:

1. 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 are related to poor cuticular development on leaves grown *in vitro* and therefore care is needed during deflasking and acclimitization in the glasshouse to achieve good establishment. Ginger is also particularly prone to sunburn (Whiley 1974), therefore the 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 needed to ensure survival and growth, production of 'seed' from micropropagated plants will be more expensive than 'seed' obtained by conventional practices.

2. During the first generation *ex vitro*, the 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 can not be used as 'seed'. This also adds significantly to the cost of 'seed' obtained from micropropagated plants.

3. Fusarium yellows is widespread in the industry and once ginger land becomes infested the disease 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 persistent nature of *Fusarium oxysporum* f. sp. *zingiberii* in the soil since the experimental site was replant ground and plants were disease-free from tissue culture. On the other hand, despite rigorous selection of conventional 'seed', rhizomes produced from this source of planting material had a much higher level with 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 it is recommended that for seed production, a nursery area should be chosen which has never produced ginger. Stringent quarantine practices are required to prevent infestation with 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 'clean' 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 'clean seed', factors must be identified that can improve rhizome size, reduce wastage and therefore improve 'seed' recovery.

In a previous study Smith and Hamill (unpublished data) found that there was a 2.6-fold decrease in rhizome yield with micropropagated plants as compared to '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. At the time it was believed 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 in the culture medium was probably not responsible for these effects. Plantlets that had been subcultured on a hormone-free medium behaved similarly though plants grown on BAP medium (TCH) consistently outperformed those grown on hormone-free medium (TCF) (Tables 1-3). There was also some indication that 'seed' recovered from TCH plants were a better source of planting material compared to the TCF plants when compared at early harvest (Tables 5-8), however even these differences were not obvious by 'seed'-harvest (Table 7).

Some other reasons that can be advanced to account for the smaller rhizomes in the micropropagated plants during their first generation ex *vitro* include:

1. Micropropagated plants have no 'seed' reserve. The plantlets at deflasking are 4-5 cm tall and weigh less than 1g whereas the 'seed' has no shoots at planting and weighs approximately 60 g. The contrasts between the two forms of planting material could not be more striking. Whiley (1980) and Okwuowulu (1988) have shown 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. It is this difference that probably accounts for the major yield differences between the micropropagated and 'seed'-derived plants. A recent study by Bhagyalakshmi *et al.* (1994), with an Indian ginger variety, also found significantly lower yields with micropropagated ginger harvested at 8 months, compared to 'seed'-derived ginger, and also attributed their difference to the fact that the micropropagated plants lacked a rhizome (seed reserves) when planted. Transplant shock was also given as a reason as 27% of the plants did not survive. Interestingly they found the yields were more comparable at 10 months as the plants continued their rapid growth under the more tropical conditions at Mysore, India (12°18'N).

2. 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 BAP in the culture medium may have been contributing to more vegetative growth in the field was not supported by our data. Another explanation may involve photoperiod. 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 16h 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 numbers' to daylength in three Japanese ginger cultivars. Okwuowulu (1988) studied the effect of 'seed' piece weight on flowering in two 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 g to 40 g. Therefore 'seed' reserve may not only have an impact on rhizome development but it also may also effect flowering.

Conclusion

A full cost-benefit analysis of the use of micropropagated ginger is needed before it can be recommended as a source of 'clean' 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. These include the need for laboratories to produce plantlets, the need for special facilities to deflask and produce the first crop of 'seed', the greater level of management required to ensure their survival and growth, the 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 that can improve 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 ginger industry.

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Figure 1. Comparison of (a) shoot height and (b) number of shoots per plant with three sources of ginger planting material. Plants were derived from 'seed' (sections of rhizome) and from micropropagated plantlets growing on either a hormone-free medium (TCF) or on a multiplication medium containing 2.5 mg l^{-1} benzylaminopurine (TCH). Values are the means of 24 replicates. Vertical bars indicate l.s.d. at $P<0.01$.

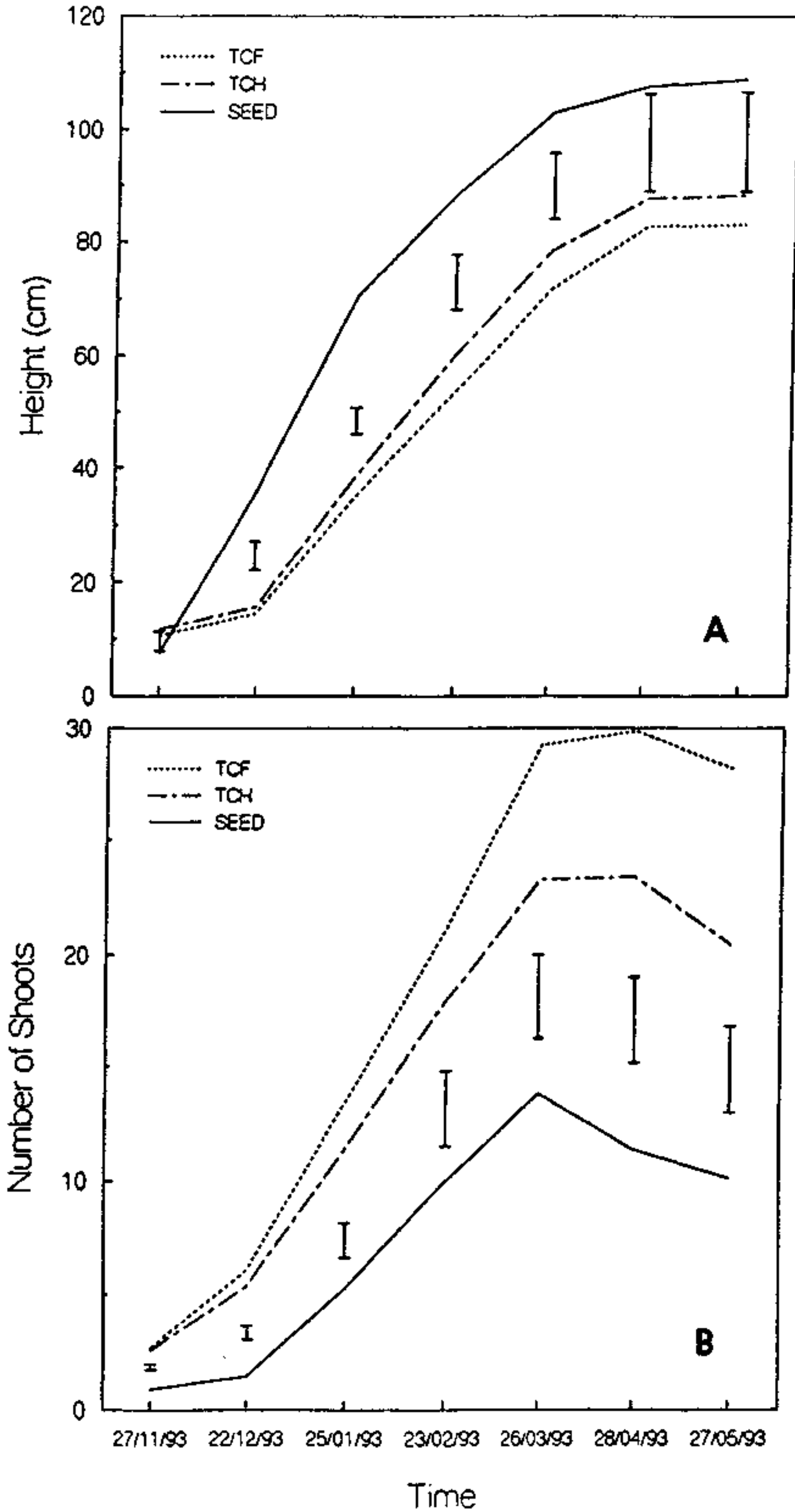

Table 1. Shoot characteristics of micropropagated and 'seed'-derived ginger plants at early-harvest.

Plants were derived from 'seed' (sections of rhizome) and from micropropagated plantlets growing on either a hormone-free medium (TCF) or a multiplication medium containing 2.5 mg l^{-1} benzylaminopurine (TCH). Data for the micropropagated plants represent the first generation *ex vitro*. Values are means of 8 replicates and data were analysed by ANOVA. Means in rows followed by the same letter(s) are not significantly different.

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

Table 2. Rhizome characteristics of micropropagated and 'seed'-derived ginger plants at early-harvest.

Plants were derived from 'seed' (sections of rhizome) and from micropropagated plantlets grown on either a hormone-free medium (TCF) or a multiplication medium containing 2.5 mg l^{-1} benzylaminopurine (TCH). Data for the micropropagated plants represent the first generation *ex vitro*. Values are means of 8 replicates and data were analysed by ANOVA. Means in rows followed by the same letter(s) are not significantly different.

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

Table 3. Rhizome characteristics of micropropagated and 'seed'-derived ginger plants at 'seed'-harvest.

Plants were derived from 'seed' (sections of rhizome) and from micropropagated plantlets grown on either a hormone-free medium (TCF) and a multiplication medium containing 2.5 mg l^{-1} benzylaminopurine (TCH). Data for the micropropagated plants represent the first generation *ex vitro*. Values are means of 24 replicates and data were analysed by ANOVA. Means in rows followed by the same letter(s) are not significantly different.

Character	Seed	TCH	TCF	l.s.d. 0.01	l.s.d. 0.05
Rhizome mass (g_{fw})	958.7[a]	462.9[b]	338.2[b]	228.7	
Root mass (g_{fw})	30.8[a]	49.3[a,b]	87.4[b]	55.5	
% Roots	3.8[a]	9.8[b]	20.0[c]	7.3	5.3
No. knobs	87.1[a]	87.2[a]	94.4[a]		
Mean knob mass (g_{fw})	10.9[a]	5.3[b]	3.6[c]	1.76	1.27
Total mass (g_{fw})	989.5[a]	512.2[b]	425.5[b]	232.6	

Table 4. 'Seed' characteristics of micropropagated and 'seed'-derived ginger plants at planting.

Rhizomes were harvested from first generation *ex vitro* micropropagated plants and from 'seed'-derived plants from Experiment 1. The micropropagated ginger was derived from plantlets grown on either a hormone-free medium (TCF) or a multiplication medium containing 2.5 mg l^{-1} benzylaminopurine (TCH). 'Seed' was prepared from these rhizomes and graded as small (35-45g), and large (55-65 g). Values are means of a sub-sample of 25 'seed' from each treatment ('seed', TCF, TCH x small, large) and data were analysed by ANOVA. Means in rows followed by the same letter(s) are not significantly different. Note that mean seed mass is lower due to some drying of the 'seed' that would have occurred during storage.

Character	Seed	TCH	TCF	l.s.d. 0.01
Seed mass (g_{fw})	46.4[a]	42.6[b]	42.1[b]	3.2
No. knobs	5.58[a]	6.88[b]	7.28[b]	0.91
Mean knob mass (g_{fw})	8.64[a]	6.48[b]	6.38[b]	1.07

Character	Seed size (g) S (35-45 g)	Seed size (g) L (55-65 g)	l.s.d. 0.01
Seed mass (g_{fw})	35.8	51.6	2.7
No. knobs	6.47	6.69	
Mean knob mass (g_{fw})	6.18	8.15	0.88

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

All plants were derived from 'seed' (sections of rhizome) harvested from Experiment 1. The original source of planting material was 'seed' and micropropagated plantlets growing on either a hormone-free medium (TCF) or a multiplication medium containing 2.5 mg l^{-1} benzylaminopurine (TCH). Values are means of 12-20 replicates and data were analysed by ANOVA. Means in rows followed by the same letter(s) are not significantly different.

Character	Seed	TCH	TCF	l.s.d. 0.05
Total shoot mass (gfw)	823.1^{a}	1183.3^{b}	927.6^{a,b}	304.4
No. shoots	20.5^{a}	29.3^{b}	24.6^{a,b}	7.5
Mean shoot mass (gfw)	39.8^{a}	41.0^{a}	37.9^{a}	
Shoot length (tallest) (cm)	78.7^{a}	84.9^{a}	83.0^{a}	

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

All plants were all derived from 'seed' (sections of rhizome) harvested from Experiment 1. The original source of planting material was 'seed' and micropropagated plantlets growing on either a hormone-free medium (TCF) or a multiplication medium containing 2.5 mg l^{-1} benzylaminopurine (TCH). Values are means of 12-20 replicates and data were analysed by ANOVA. Means in rows followed by the same letter(s) are not significantly different.

Character	Seed	TCH	TCF	l.s.d. 0.01
Rhizome mass (g_{fw})	702.3^{a}	950.9^{a}	723.0^{a}	
'Choice'-grade mass (g_{fw})	285.6^{a}	358.3^{a}	278.3^{a}	
% 'Choice'-grade	41.1^{a}	37.9^{a}	39.0^{a}	
Root mass (g_{fw})	9.9^{a}	25.5^{b}	15.8^{a,b}	14.5
% Roots	1.36^{a}	2.54^{a}	3.71^{a}	
No. knobs	74.0^{a}	99.9^{a}	78.3^{a}	
Mean knob mass (g_{fw})	9.55^{a}	9.56^{a}	8.86^{a}	
Total mass (g_{fw})	712.2^{a}	975.9^{a}	738.8^{a}	
Shoot:root (rhizome) ratio	1.13^{a}	1.20^{a}	1.52^{a}	

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

All plants were derived from 'seed' (sections of rhizome) harvested from Experiment 1. The original source of planting material was 'seed' and micropropagated plantlets growing on either a hormone-free medium (TCF) or a multiplication medium containing 2.5 g l^{-1} benzylaminopurine (TCH). Values are means of 20-40 replicates and data were analysed by ANOVA. There were no significant differences between treatments.

Character	Seed	TCH	TCF
Rhizome mass (g_{fw})	1167.0	1188.0	1391.2
Root mass (g_{fw})	19.5	26.8	21.5
% Roots	1.75	2.20	1.66
No. 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