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Ginger (*Zingiber officinale*) autotetraploids with improved processing quality produced by an *in vitro* colchicine treatment

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Abstract. Ginger autotetraploids were produced by immersing shoot tips in a 0.5% w/v colchicine, 2% v/v dimethyl sulfoxide solution for 2 h. Stomatal measurements were used as an early indicator of ploidy differences in culture with mean stomata length of tetraploids (49.2 μm) being significantly larger than the diploid (38.8 μm). Of the 500 shoot tips treated, 2% were characterised as stable autotetraploid lines following field evaluation over several seasons. Results were confirmed with flow cytometry and, of the 7 lines evaluated for distinctness and uniformity, 6 were solid tetraploid mutants and 1 was a periclinal chimera. Significant differences were noted between individual tetraploid lines in terms of shoot length, leaf length, leaf width, size of rhizome sections (knob weight) and fibre content. The solid autotetraploid lines had significantly wider, greener leaves than the diploids, they had significantly fewer but thicker shoots and, although 'Queensland' (the diploid parent from which the tetraploids were derived) had a greater total rhizome mass at harvest, its knob size was significantly smaller. From the autotetraploid lines, one line was selected for commercial release as 'Buderim Gold'. It compared the most favourably with 'Queensland' in terms of the aroma/flavour profile and fibre content at early harvest, and had consistently good rhizome yield. More importantly it produced large rhizome sections, resulting in a higher recovery of premium grade confectionery ginger and a more attractive fresh market product.

Introduction

The diploid ($2n = 22$) cultivar, 'Queensland', is favoured for the production of confectionery ginger in Australia and it is estimated that 40% of the world's confectionery ginger products are based on this single cultivar (G. O'Brien pers. comm.). Unfortunately much of the rhizome that is harvested has small rhizome sections or knobs that are unsuitable for processing into the premium grade, export product.

The use of colchicine to produce polyploid plants with larger structural organs is well known. In ginger this strategy is particularly attractive as the crop is vegetatively propagated and any genetic improvements that are made to yield or quality can be preserved over successive generations. Ramachandran (1982) treated sprouting buds on ginger rhizomes *in vivo* with 0.25% (w/v) colchicine solution and of the 90 buds treated, 4 produced solid, non-chimeral tetraploids. Ramachandran and Chandrasekharan Nair (1992) evaluated these tetraploids and found that there was an overall increase in size of plant parts including the leaves, floral parts and the rhizome. Unfortunately, oil content was lower in the tetraploid than in the original diploid cultivar. *In vitro* induction of autotetraploids is a more attractive strategy because it is possible to effectively treat larger numbers of explants and to rapidly produce disease-free and pest-free planting material for field evaluation (Hamill *et al*. 1992). Smith and Hamill (1997) immersed ginger shoot tips in a 0.5% (w/v) colchicine solution with 2% (v/v) dimethyl sulfoxide for 2 h to produce stable autotetraploid lines. Adaniya and Shirai (2001), on the other hand, favoured an 8-day treatment on solid agar-based culture medium containing 0.2% (w/v) colchicine. Their preliminary results also indicate a larger rhizome was produced and the gingerol content of the tetraploid lines were higher than those of their diploid counterparts.

The present study reports on a technique to increase rhizome size by the *in vitro* induction of ginger autotetraploids from the 'Queensland' cultivar and the methods used in the early detection of these lines. Field performance of the tetraploid lines was investigated over several seasons and an assessment made of their suitability for processing into a premium-grade confectionery product.

Materials and methods

Establishment of cultures

A rhizome of ginger (*Zingiber officinale* Rosc.) cv. 'Queensland' was supplied by Buderim Ginger Limited from a plant exhibiting superior characteristics. To initiate cultures, the rhizome was surface sterilised with 1% sodium hypochlorite for 2 min and then stored in the dark at ambient temperature until it began to sprout. Emerging buds

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(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 with the pH adjusted to 6.0. Shoots proliferated on this medium at the rate of 4–5 per month when 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 about 80 μmol quanta/m^2.s. The formation of a good root system was also facilitated by the same medium and plantlets could be readily deflasked and established in the glasshouse.

Colchicine treatment

The procedure for *in vitro* induction of autotetraploids was after the method of Hamill *et al*. (1992). Explants, with the roots trimmed and shoots removed 4–5 mm above the shoot tip, were placed onto hormone free MS media until they were showing signs of active growth and were free from contamination (1 week), then placed in 25 mL liquid MS medium in 125 mL Erlenmeyer flasks containing 0.5% (w/v) colchicine and 2% (v/v) dimethyl sulfoxide (DMSO). The explants were transferred to the colchicine solution at the commencement of the light cycle (incubation conditions as above) as cell division may be synchronised by light stimulus and more cells become active after exposure to light following a dark period (Iyer and Randhawar 1965). Flasks were placed on an orbital shaker at 100 rpm and after 2 h the explants were removed and washed thoroughly on a sieve with 500 mL of sterile distilled water before being placed onto solid MS medium. 500 explants were treated in this manner.

Selection of autotetraploids

Autotetraploid ginger was first selected on the basis of altered morphology, particularly the size of the stomata. Stomata were measured from an imprint of the leaf surface obtained by painting clear fingernail polish on the leaf, allowing it to dry and peeling it off with the aid of clear adhesive tape. The length of 10 stomata was measured from the upper surface of each leaf at ×400 magnification. Putative tetraploids were grouped and the chromosome number was determined from a root tip squash. Root tips were fixed in Farmer's fixative (glacial acetic acid:95% ethanol, 1:2) for a minimum of 72 h. Roots were rinsed in water and softened in 5 mol/L HCl for 25 min at room temperature then squashed and stained with an altered form of carbol fuchsin (Martens and Reisch 1988).

Establishment and design of field trials

The procedures for deflasking, field establishment and harvesting have been described previously (Smith and Hamill 1996). Seed-pieces (sections of the rhizome used for planting) for controls were provided by Buderim Ginger Limited. Initial field trials were conducted on the Australian Golden Ginger Experimental Farm near Kandanga (26°10′S) in a brown, clay-loam soil. The first generation of *ex vitro* plants were grown under shade to reduce plant loss during the hot, dry conditions that are frequently experienced in late spring and early summer. The micropropagated ginger was planted on 20 October 1992 in a randomised block design with 8 replicates and 3 treatments (diploid seed, micropropagated diploid, micropropagated tetraploid). At this stage all of the tetraploid lines, while separately labelled, were treated as a single population for comparative purposes. Early harvest took place on 5 April 1993 and corresponded with a period of maximum recovery of 'choice' grade ginger. Choice grade ginger (used for confectionary) has commercially acceptable levels of fibre when 35–45% by weight of the rhizome is classed as 'fibre-free' (Whiley 1980). Plants that were not harvested for data collection were held over for a seed harvest on 1 September 1993. Rhizomes were harvested and cut into seed-pieces that varied in size from 35 to 65 g. Seed was treated for 10 min with 1 g/L Benlate (0.5 g/L benomyl) and air-dried before being stored in a cool, dry place until planting in October. Data were analysed by ANOVA.

For the following 6 vegetative cycles, the diploid and tetraploid lines were grown in the field, first at Kandanga, and subsequently at Maroochy Research Station (26°37′S). At each stage of this 'bulking-up' period, poor performing and unthrifty plants were discarded. Only the best material from each line was carried through to the next generation. During October 1999–May 2000 and September 2000–April 2001 plants were grown at Maroochy Research Station using commercial farming practices (Smith and Hamill 1996). Plants were grown in a 3-row bed with 20 cm between plants along the row and 30 cm between rows. A randomised complete bock design was used with 30 replicates each of 6–7 tetraploid lines, as well as diploid 'Queensland', from which the tetraploids were derived, and diploid 'Canton'. 'Canton' was chosen as a comparator in the trials because it is the only cultivar grown in Australia with a large rhizome that could conceivably be used to produce a premium-grade confectionery product. During the 2001 harvest 4 plants were collected at 5 periods between 15 February and 1 March, which coincided with the early harvest for factory processing. They were commercially assessed for fibre content and percentage of 'choice' grade ginger at Buderim Ginger's factory. The final destructive harvest in May 2000 and April 2001 coincided with the attainment of maturity for the crop and when maximum yields were attained. This was required under the UPOV (1996) guidelines for the conduct of tests for distinctness, uniformity and stability.

Measurements

Plants were pulled from the ground at harvest and hosed to remove soil before the following traits were measured: number of shoots, length of shoot (cm), leaf length (mm), leaf width (mm), number for leaves per stem, shoot diameter (mm), total shoot fresh weight (g_{fw}), rhizome fresh weight (g_{fw}), root fresh weight (g_{fw}) and number of rhizome sections or knobs. From the rhizome weight and number of knobs, mean knob size was calculated. This character is important, as large knobs are favoured during factory processing and for the sale of premium grade confectionery ginger, as well as for the fresh market. The recovery of choice (fibre-free) 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 areas (cm^2) per shoot determined. From these measurements total leaf area per plant could be estimated. The intensity of green colour in the leaves was rated on a scale of 1–7 where 3 is light green, 5 is medium, and 7 is dark green (UPOV 1996).

Linear mixed models were used to analyse the data for each trait at each harvest (May 2000 and April 2001). The initial model for each trait included a fixed variety term and a random term to model the block design factor. The plot errors were modelled using spatial methods of analysis (Gilmour *et al*. 1997). The method of generalised least-squares was used for the fixed effects and best linear unbiased prediction (BLUP) was used for the random effects. The restricted maximum likelihood (REML) method (Patterson and Thompson 1971) was used to estimate variance components for the random effects. All analyses were carried out in SPLUS using samm (Butler *et al*. 2000).

Cytometric/ploidy analyses

The ploidy of the ginger plants from the 1999–2000 field trial was determined by analysing leaf sheath, shoot and rhizome samples using flow cytometry. Leaf sheaths, shoots and rhizome samples from diploid 'Queensland' were used as an external control. The samples were sent to the Plant Improvement Unit of the Institute for Tropical and Subtropical Crops, Nelspruit, South Africa, who provide a flow cytometry service.

Samples for flow cytometric analyses were prepared according to Dolezel *et al*. (1994). Intact nuclei were released from cut surfaces from

the leaf sheath, shoot and rhizome respectively by chopping the sample with a razor blade in a drop of LB01 buffer (Dolezel *et al*. 1989). An additional 300 µL of LB01 buffer (15 mol/L Tris, 2 mol/L Na_2EDTA, 80 mol/L KCl, 20 mol/L NaCL, 0.5 mol/L spermidine, 15 mol/L mercaptoethanol, 0.1% Triton X-100 at pH 7.5) was added to the sample and the released nuclei were filtered through a 50 µm nylon mesh. The nuclei were stained with DAPI (4′,6-diamino-2 phenylindole) to a final concentration of 2 µg/mL and analysed immediately. The leaf sheath, shoot and corm of each sample were analysed separately. A more precise estimation of ploidy was made thereafter by combining the rhizome sections of each sample with a rhizome section of the diploid control. The ratio of the G_1 peak position of the test specimen/reference was then used to calculate the DNA ploidy.

The relative fluorescence intensity of the samples was analysed using a PA Flow cytometer (Partec GmbH, Germany). Between 10000 and 110000 nuclei were analysed per sample. A diploid ginger sample from South Africa was used as a reference to calibrate the arbitrary units or channels. The flow cytometer was adjusted so that the peak, representing the diploid/G_1 nuclei was located at channel 100. This setting was kept constant and the remaining samples were characterised by the position of their peak.

Analyses of aroma, flavour and pungency

Aroma, flavour and pungency were measured from a subsample of rhizomes randomly selected from each block during both harvests. Measurements were made by the Department of Primary Industries and Fisheries (DPIF), Queensland, who provide this service. Aroma was assessed by measuring total volatile component content. This was achieved using headspace gas chromatography–mass spectrometry analysis on each macerated rhizome sample (Miyazawa and Kameoka 1988; Tanabe *et al*. 1991). Oil for analysis of flavour and pungency was extracted from raw ginger rhizome samples using a supercritical fluid extraction–CO_2 unit (Suprex Prepmaster, Pittsburg, PA) set at 40075 kPa and 67°C (Pham 1996). Flavour assessment was carried out using direct injection GC-MS of the extracted oils (Harvey 1981; MacLeod and Pieris 1984; Chen and Ho 1988). Pungency was assessed using high performance liquid chromatographic analysis for the detection of the total pungents in the extracted oil (Pham 1996). Finally, percentage oil content was determined on a subsample (2 g) of raw rhizome by hydrolytic digestion with concentrated ammonium hydroxide for 10–15 min in a mojonnier flask at 65°C and extracted with diethyl ether. The ether extract was decanted and evapourated at 70°C to yield a viscous, yellow oil. Oil content was expressed as g/100 g rhizome.

Results

Explants treated with colchicine were initially slower growing than untreated control plants. Mortality was 3% for the controls but up to 89% for the colchicine-treated plants after 8 weeks' culture. This compared with 48% mortality in diploid bananas given the same colchicine treatment (Hamill *et al*. 1992). Of the plants that survived the colchicine treatment, some had broader and greener leaves. Stomata size differed significantly between the micropropagated diploid and tetraploid ginger (Fig. 1). The tetraploid had larger stomata in the range of 39.4–60.8 µm with a mean of 49.2 µm while the diploid stomata length ranged from 29.1 to 48.6 µm with a mean of 38.8 µm (P<0.01). There was no variation in stomata length between diploid plants from different culture bottles. Subsequent chromosome counts revealed that 27% of the surviving plants were autotetraploid. Therefore of the 500 shoot tips treated, 15 were confirmed as autotetraploids. The ginger autotetraploids continued to grow and proliferate in culture and the multiplication rate of the autotetraploids was similar to the diploids after several more months of culture.

The 15 autotetraploid plants selected in culture were micropropagated for a further 4 subculture cycles. Rapid *in vitro* propagation following colchicine treatment has been shown to increase the percentage of solid, non-cytochimeral polyploids obtained in vegetatively propagated crops (Broertjes and van Harten 1988). Nevertheless 3 of the tetraploid lines reverted to the diploid state.

Plants were established in the field and stomatal measurements were re-examined. The field-grown ginger showed similar trends to the *in vitro* material (Fig. 1). The mean stomata length of the autotetraploid was 50.8 µm compared with 36.7 µm for the diploid (P<0.01). However it was the contrasts in shoot and rhizome characters between the micropropagated diploid and autotetraploid ginger that were the most striking (Table 1). The autotetraploids had fewer but larger stems and leaves, and were similar in many respects to the diploid ginger propagated from conventional seed-pieces. The micropropagated tetraploids also produced a significantly (P<0.01) larger rhizome than the micropropagated diploid and, although the number of knobs was similar, the average knob size was significantly (P<0.01) greater for the autotetraploid ginger. Again the rhizome of the micropropagated tetraploid ginger was similar to the conventionally propagated diploid except for

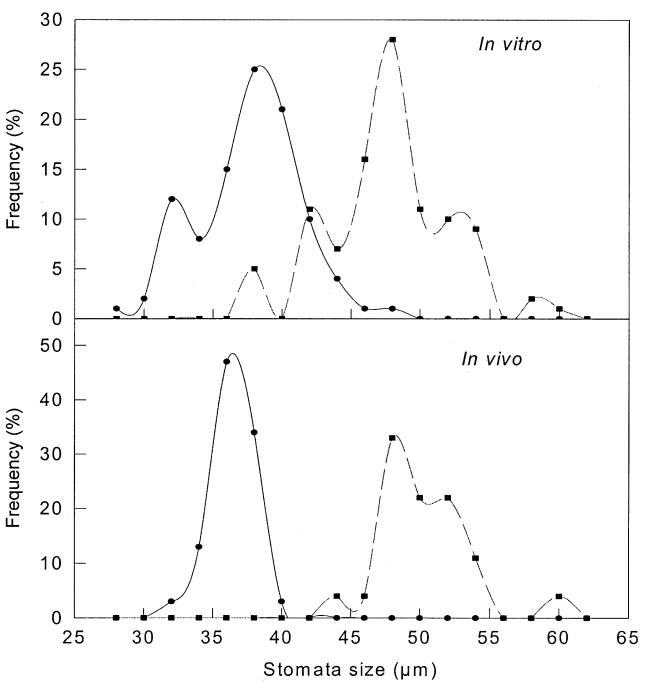

Figure 1. Stomata size distribution of diploid (●) and autotetraploid (■) ginger from (*a*) *in vitro* plants and (*b*) field-grown plants.

Table 1. Shoot and rhizome characteristics of first generation *ex vitro* micropropagated and seed-derived ginger plants at early harvest during 1993

Values are means of 24 replicates; means within each row followed by the same letter are not significantly different at $P = 0.01$

Characteristic	Diploid 'seed'[A]	Micropropagated diploid[B]	Micropropagated tetraploid[B]	l.s.d. ($P = 0.01$)	l.s.d. ($P = 0.05$)
No. of shoots	9.3a	27.0b	14.5ab	—	12.6
Mean shoot length (cm)	86.6a	57.2c	70.8b	20.2	14.1
Mean shoot mass (g_{fw})	62.3a	18.8b	52.5a	19.5	—
Mean leaf area (cm^2)	51.7a	28.7b	53.8a	12.9	—
Leaf area per shoot (cm^2)	846.3a	416.5c	677.4b	203.6	139.9
Plant leaf area (cm^2)	9992a	11288a	9818a	—	—
Mean stem mass (g_{fw})	43.1a	17.6b	42.8a	11.8	—
Mean leaf mass (g_{fw})	17.6a	8.0b	17.6a	4.7	—
Rhizome mass (g_{fw})	635.5a	175.3b	506.3a	444.7	309.6
Root mass (g_{fw})	15.0a	67.8a	107.8a	—	—
No. of knobs	58.0a	49.8a	57.5a	—	—
Mean knob mass (g_{fw})	10.9a	3.4b	8.9a	4.4	—

[A]Plants derived from seed-pieces of 'Queensland'.
[B]Plants derived from micropropagated 'Queensland' or autotetraploids induced from 'Queensland'.

the greater mass of roots that seems to be a characteristic of the first generation of micropropagated plants grown in the field (Smith and Hamill 1996).

Although the micropropagated diploid and conventionally propagated diploid are significantly different during the first generation *ex vitro*, Smith and Hamill (1996) have shown that seed-pieces recovered from this first crop later grow on to produce plants that are similar to plants grown from seed-pieces. This is despite the fact that the size of seed-pieces recovered from the micropropagated ginger's first generation crop is generally smaller than normally used for planting. After 7 vegetative cycles carried out in the field, 7 autotetraploid lines (33, 67, C24, C42, C5, C52, C7) were selected for more thorough examination with the ultimate aim to commercially release one or more of these lines to the ginger industry.

During the 2000 harvest the tetraploid lines had significantly wider, greener leaves than the diploids, they had significantly fewer but thicker shoots and, although 'Queensland' had a greater total rhizome mass at harvest, its knob size was significantly ($P<0.05$) smaller (Table 2). Interestingly the 'Canton' cultivar had characteristics intermediate between the tetraploids and the 'Queensland' cultivar. It was also interesting to note that flow cytometry analysis revealed that one autotetraploid line, C7, was a periclinal chimera with the analysis of the leaf sheaths, stems and rhizomes showing $2n + 4n$ peaks (Fig. 2). All other tetraploid lines were solid $4n$ mutants (Fig. 3).

The autotetraploid lines, with the exception of C7, were evaluated over another season (2000–01) and compared with the diploid cultivars. Again the tetraploid lines differed significantly ($P<0.05$) from the 'Queensland' cultivar in relation to the number of shoots, leaf length, leaf width,

Table 2. Shoot and rhizome characteristics of 7 selected tetraploid lines and 2 diploid cultivars harvested at maturity during 2000

Values are means of 30 replicates; means within each column followed by the same letter are not significantly different at $P = 0.05$

Tetraploid line or cultivar	No. of shoots	Shoot length (cm)	Leaf length (mm)	Leaf width (mm)	No. of leaves per stem	Intensity of leaf colour[A]	Shoot diameter (mm)	Rhizome mass (g_{fw})	No. of knobs	Knob mass (g_{fw})
33	7.47ab	89.5e	26.8ab	3.84d	25.1c	5.90c	14.5bc	1047.0b	66.7a	16.4cd
67	7.87ab	81.4bc	26.0a	3.45b	23.4bc	5.90c	13.7b	906.2ab	58.7a	16.1cd
C24	6.44a	87.0de	28.2c	3.92d	22.2bc	5.90c	14.4bc	873.6ab	53.4a	16.8d
C42	7.57ab	88.5de	27.9c	3.86d	22.0abc	5.87c	15.1c	954.8ab	61.6a	16.0cd
C5	7.57ab	84.8cd	27.8bc	3.95d	21.8ab	5.90c	14.5bc	879.5ab	58.8a	15.2bd
C52	8.42b	89.1de	27.9c	3.86d	23.9bc	5.87c	15.0c	1016.4b	66.5a	15.9cd
C7	7.32ab	76.6a	28.6c	3.63c	19.0a	5.93c	13.4b	754.2a	54.3a	13.6b
'Canton'	10.83c	80.0ab	26.5ab	2.94a	21.5ab	5.53b	10.8a	1018.0b	67.0a	14.9bc
'Queensland'	16.77d	86.0de	28.2c	3.07a	21.8ab	5.18a	11.5a	1428.1c	163.7b	8.9a
l.s.d. ($P = 0.05$)	1.77	4.4	1.3	0.18	3.1	0.22	1.1	209.8	14.0	1.7

[A]Intensity of green colour in leaves was rated on a scale of 1–7 (3, light green; 5, medium; 7, dark green) (UPOV 1996).

intensity of leaf colour, shoot diameter, number of knobs and knob weight (Table 3). The 'Canton' cultivar was also intermediate in several characters. In regards to the autotetraploid lines, 33, 67 and C42 produced consistently high rhizome yields with excellent knob size over both seasons. However early harvest results in the factory revealed that C42 was the most fibrous of the tetraploid lines and, as a result, percentage recovery of 'choice' grade ginger was lowest from this line (Table 4).

Analysis for aroma, flavour and pungency (data not shown) showed that there was variation between lines with some lines such as C24 and C52 lacking in overall taste and C5 and 67 having higher pungency per rhizome mass. Of all of the autotetraploid lines, 33 compared the most favourably with 'Queensland' in terms of the aroma/flavour profile.

Discussion

In vitro induction of ginger autotetraploids from the cultivar, 'Queensland', succeeded in developing plants with

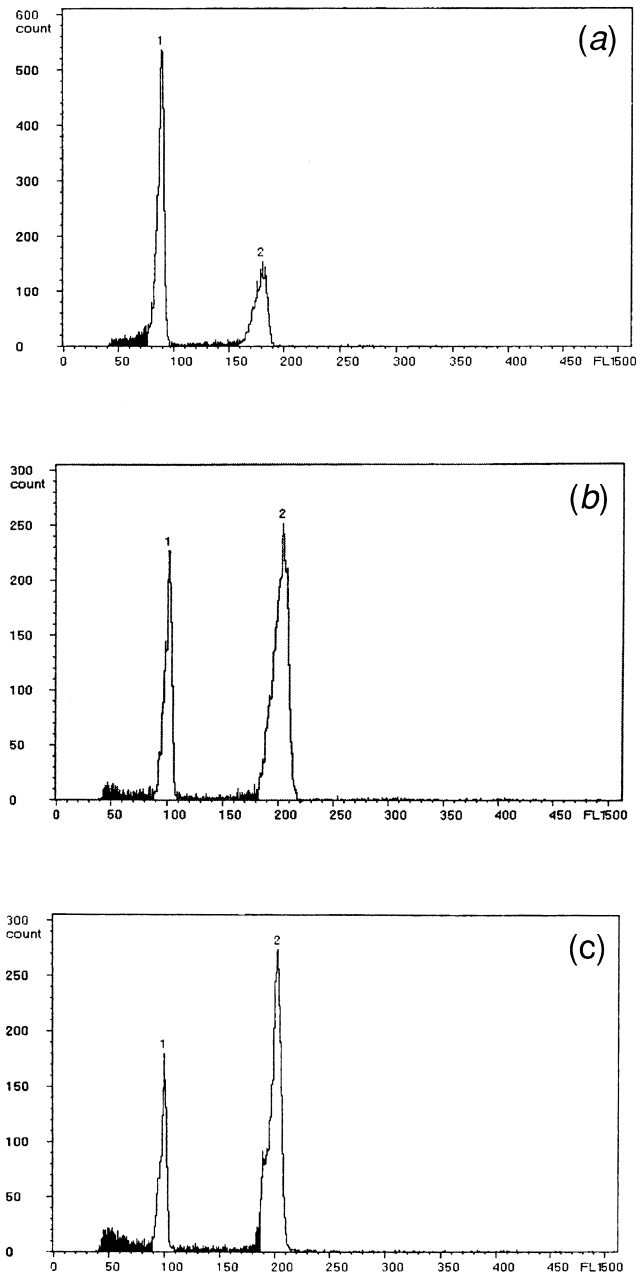

Figure 2. Histograms with nuclei counting results and peak analysis of line C7 samples obtained from (*a*) leaf sheaths, (*b*) stems and (*c*) rhizomes (peak 1 = 2*n*, peak 2 = 4*n*).

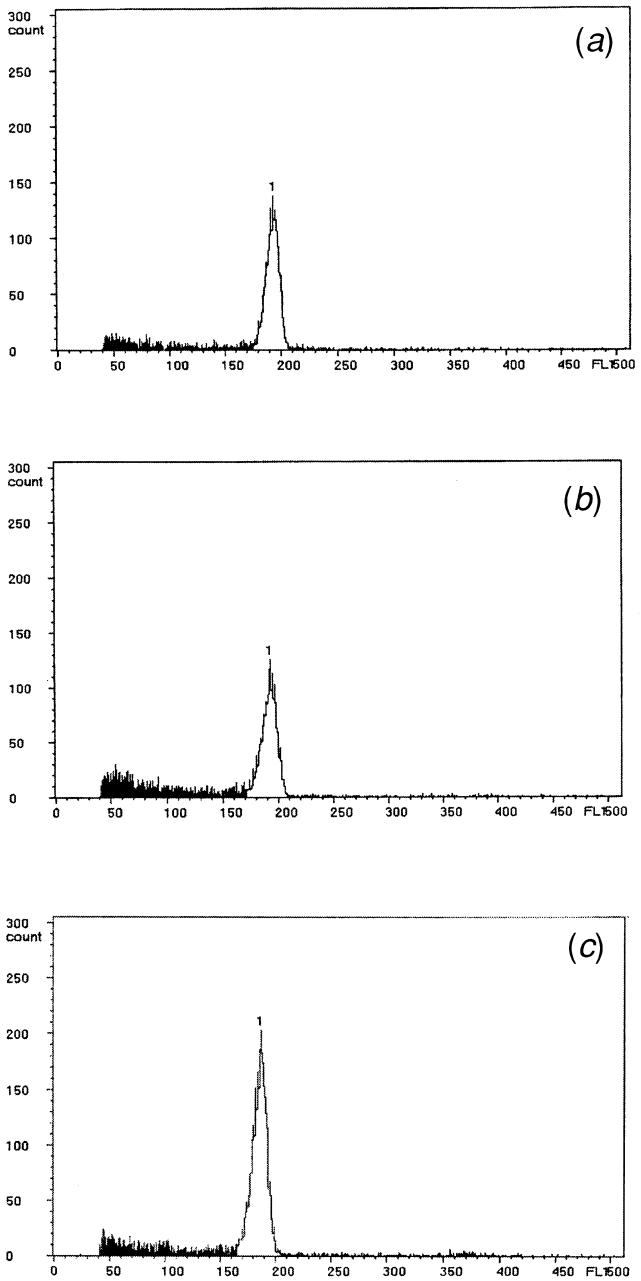

Figure 3. Histograms with nuclei counting results and peak analysis of line 33 samples obtained from (*a*) leaf sheaths, (*b*) stems and (*c*) rhizomes (peak 1 = 4*n*).

Table 3. Shoot and rhizome characteristics of 6 selected tetraploid lines and 2 diploid cultivars harvested at maturity during 2001

Values are means of 30 replicates; means within each column followed by the same letter are not significantly different at $P = 0.05$

Tetraploid line or cultivar	No. of shoots	Shoot length (cm)	Leaf length (mm)	Leaf width (mm)	No. of leaves per stem	Intensity of leaf colour[A]	Shoot diameter (mm)	Rhizome mass (g_{fw})	No. of knobs	Knob mass (g_{fw})
33	7.30ab	78.5b	26.3c	3.66c	21.7	4.33b	13.1bc	799.0	47.4abc	17.0bc
67	7.03a	69.5a	24.9b	3.19b	22.4	4.39b	13.3bc	821.6	46.2ab	18.4c
C24	6.08a	78.4b	25.8bc	3.40bc	22.8	4.63bc	13.3bc	651.0	40.6a	16.6bc
C42	6.71a	85.6c	26.4c	3.59c	24.4	5.08c	14.5c	958.2	51.1ab	19.1c
C5	7.75ab	77.3b	26.4c	3.64c	20.9	4.75bc	12.9bc	828.8	54.8bc	15.3b
C52	6.92a	76.1b	25.8bc	3.57c	22.2	4.17ab	12.8b	753.9	44.7ab	17.1bc
'Canton'	9.62b	68.0a	23.9ab	2.68a	22.4	4.29ab	10.5a	885.8	60.6c	14.5b
'Queensland'	12.63c	66.0a	23.5a	2.48a	20.9	3.63a	9.5a	812.4	82.7d	9.9a
l.s.d. ($P = 0.05$)	2.49	5.8	1.1	0.30	n.s.	0.69	1.6	n.s.	13.4	3.0

[A]Intensity of green colour in leaves was rated on a scale of 1–7 (3 = light green; 5 = medium; 7 = dark green) (UPOV 1996).

good yields and larger rhizome sections while still retaining many of the characters so important in producing a quality confectionery product. Of the autotetraploid lines examined in detail in field and factory trials, line 33 satisfied all of the selection criteria and has been released to the industry as 'Buderim Gold' (Smith and Hamill 2002).

One of the important findings from this work was the need to carefully evaluate each tetraploid line individually, as significant differences were noted in several characters. No other studies with autotetraploid ginger lines have reported individual differences between lines generated from a common parent. It is also important in ginger not to place too much importance on rhizome characteristics of the first generation's crop following micropropagation as plants typically produce smaller rhizomes with excess root development and lower yields of oils and oleoresins (Bhagyalakshmi *et al*. 1994; Smith and Hamill 1996). Subsequent cycles of growth and selection are necessary to 'stabilise' lines (i.e. 2 or more vegetative cycles where the crop is grown from seed-pieces rather than from micropropagated plants eliminates earlier problems with poor yields). In addition, mericlinal and sectorial chimeras can be eliminated and stable autotetraploid lines, either solid or periclinal chimeras, can be grown for evaluation.

Table 4. Early harvest characteristics of 6 selected tetraploid lines and 2 diploid cultivars during factory evaluation of rhizomes at Buderim Ginger Limited

Values are means of 20 replicates; means within each column followed by the same letter are not significantly different at $P = 0.05$

Tetraploid line or cultivar	Choice grade mass (g_{fw})	Second grade mass (g_{fw})	Total mass (g_{fw})	Percentage choice
33	196.1	241.7	437.7	44.7ab
67	195.3	249.6	444.9	43.5ab
C24	192.5	241.8	444.3	43.6ab
C42	172.4	244.7	417.0	41.0a
C5	171.6	204.2	375.8	47.3b
C52	173.5	191.8	355.8	48.2b
'Canton'	217.2	226.3	443.6	48.4b
'Queensland'	195.5	244.2	439.7	44.8ab
l.s.d. ($P = 0.05$)	n.s.	n.s.	n.s.	6.0

Of the 15 autotetraploid lines produced *in vitro*, 3 were unstable and reverted to the diploid state. One line, C7, was a stable periclinal chimera but had low yields and poor plant structure. Of the remaining solid autotetraploids, C24 gave consistently low yields and C42, while consistently high yielding, had the most fibrous rhizome at early harvest. A closer examination of shoot characteristics also revealed significant differences between lines. For instance 67 produced smaller shoots and leaves compared with 33 and C42. Apart from mutations that may have arisen during chromosome doubling, the variation could also be accounted for by culture-induced mutations that lead to enhanced genetic variation among plants regenerated from *in vitro* culture. It is a widespread phenomenon and has been called somaclonal variation (Larkin and Scowcroft 1981; Scowcroft 1984). The fact that individual bud lines had been micropropagated for several vegetative cycles, both before and after colchicine treatment, provides ample opportunity for somaclonal variation to occur. Damasco *et al*. (1998) were able to show that a common somaclonal variant of banana arose as early as the fourth subculture after initiation of shoot tips.

All of this reinforces the UPOV (1996) guidelines for the need to evaluate lines or cultivars over 2 similar growing periods with 2 consecutive plantings, the second being a replanting with the same plant material. It is advisable to conduct trails at one location and each trial should include a minimum of 30 plants in a randomised, complete block design.

Our *in vitro* induction technique using 0.5% (w/v) colchicine and 2% (v/v) DMSO applied for 2 h in liquid culture was effective in generating solid, non-chimeral tetraploid plants with a conversion frequency of 2%. The

procedure was applied directly from the work of Hamill *et al*. (1992) working with bananas and was not optimised for the ginger tissue culture system. Adaniya and Shirai (2001), on the other hand, examined the effect of 0.2% (w/v) colchicine applied in either liquid or solid medium over a 14-day period. Their results showed that solid medium was more efficient than liquid medium for tetraploid induction and that, irrespective of the type of medium, an 8-day treatment gave more solid, non-chimeral tetraploids than any other period of treatment. Their best treatment on solid medium gave a conversion frequency as high as 30%, compared with the best treatment on liquid medium of 10%, however mortality was decreased on a liquid medium. The advantage of an *in vitro* system over an *in vivo* system is that large numbers of explants can be treated quickly and efficiently, and once tetraploids are identified *in vitro*, they can be rapidly multiplied for evaluation in the field.

This study resulted in the commercial release of a new variety of ginger for the Australian industry and demonstrated the utility of *in vitro* induction of autotetraploid lines. It can be applied to any ginger variety and we are currently targeting cultivar 'Jamaican' that has high oils and oleoresins, and high α-zingiberene, but very poor rhizome size (data not shown).

Production issues have arisen with the release of a new cultivar with significantly larger rhizome sections and relate mainly to larger seed-pieces used for planting. Further work is needed to improve germination and to increase shoot density within the crop, so necessary in weed control and protection of young shoots from sunburn (Whiley 1974). Experiments with planting density (Whiley 1981) have shown that it is possible to increase yield with higher density plantings while not having a major impact on knob size, which is critical for factory processing. Using a cultivar that produces significantly larger knobs from the outset means that industry concerns of a reduction of knob size with high-density plantings is probably not an issue. It is equally important with a new cultivar to understand its rhizome phenology as the early harvest period is characterised by a change from growth by extension to growth by starch accumulation (Sanewski 2002). In other words if the crop is harvested too early, tonnage will be low; but if harvested too late, the percentage of choice grade ginger will be reduced due to fibre development as the rhizome matures. Research is needed to answer these questions with 'Buderim Gold' and to develop suitable production practices that satisfy both growers and processors alike.

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