VG403
The development of tetraploid ginger varieties

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VG403

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Final Report (VG403)

The Development of Tetraploid Ginger Varieties

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THE DEVELOPMENT OF TETRAPLOID GINGER VARIETIES

HRDC PROJECT NO. VG403

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1. Final Report Summary

a) Industry Summary:

In southeasten Queensland, ginger is grown in an area (about 150 ha) centred on Yandina. Each year approximately 5600 t of rhizome is processed for a value of A$ 13.5 m. Buderim Ginger Ltd has one of the world's largest ginger factories and processes half of the world's supply of confectionary ginger. Profitability of the Australian ginger industry could be enhanced by improvement of cv. Queensland which has unique properties favoured during factory processing. Rhizomes bearing large knobs are preferred for processing into high quality confectionary ginger. Because the knob size is variable, and small knobs can not be satisfactorily used for 1st-grade confectionary ginger, rhizomes with small knobs are processed for less profit. Approximately 70% of the harvested ginger is currently used for less profitable operations.

Ginger has 22 chromosomes and is a diploid. One possible way to increase rhizome knob size would be to produce a tetraploid ginger plant with twice as many chromosomes because in some plant species a reasonable increase in numbers of genomes is accompanied by an increase in cell size and larger organs. Potato is a well known tetraploid plant. We successfully developed a technique for producing banana tetraploids 6 years ago by immersing tissue cultured shoot tips in a colchicine solution for 2 hours. Colchicine is an alkaloid obtained from the autumn crocus well known for its ability to double chromosome number in plants. The procedure we used for banana was followed for ginger .

Tetraploid 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. Putative tetraploids were grouped and the chromosome number was determined from a root-tip squash.

Tetraploid plantlets were subcultured over a 8 month period before being deflasked and acclimatised in a glasshouse. After 7 weeks the plants were taken to the Australian Golden Ginger Experimental Farm near Kandanga and established in the field under 50% shade. The micropropagated ginger was planted on 20 October and the early harvest took place on 5

April when the flower heads emerged. This corresponded with a period of maximum recovery of 'choice' grade ginger.

The contrast between the micropropagated diploid and tetraploid ginger was striking. The tetraploid had fewer but larger stems and leaves, and was similar in many respects to the diploid ginger propagated from conventional 'seed'-pieces (sections of the rhizome). The situation below ground also revealed similar differences. The tetraploid produced a larger rhizome and, although the number of knobs was similar to the micropropagated diploid, the average knob size was greater for the tetraploid ginger.

The Future

Although the micropropagated diploid and conventionally propagated diploid are significantly different during the first generation out of culture, we have shown that seed-pieces recovered from this first crop later grow on to produce plants that are similar in all respects. This is despite the fact that the size of seed-pieces recovered from the micropropagated ginger's first generation crop is generally smaller.

If the differences between the diploid and tetraploid ginger continue into the second generation's crop then we can expect to see an improvement in recovery of rhizomes with larger knobs.

Plant material from the tetraploid selections will be multiplied for further evaluation in the field and in the factory. To remain competitive and profitable, continued effort must be made in improving yield and recovery of premium-grade ginger.

b) Technical Summary:

In vitro ginger shoot tips were immersed in a 0.5% w/v colchicine solution with 2% v/v DMSO for 2h under aseptic conditions. Autotetraploids were first selected on the basis of changes in general morphology - broader,greener leaves and thicker stems and roots. They were further screened on the basis of larger stomatal size compared to the micropropagated diploid selections and were later confirmed by chromosome counts from root tip squashes. Although some reversion to the diploid state has occurred, a selection of autotetraploid lines has been recovered that are believed to be more stable. The autotetraploids examined after the first generation *ex vitro* had larger stems, leaves and rhizomes than the micropropagated diploid ginger. More importantly the knob size, which is important in factory processing of confectionary ginger, was larger in the autotetraploids. Plant material from the autotetraploid selections is currently being multiplied for further evaluation.

c) Publication:

Smith, MK and SD Hamill (1997) The development of tetraploid ginger varieties. In, 'Tissue Culture: Towards the Next Century. Proceedings of Vth International Association for Plant Tissue Culture (Australian Branch) conference, Gatton, Queensland, 2-6 December, 1996'. pp.221-223. (Eds. A Taji and R Williams). University of New England Press: Armidale, NSW.

The development of tetraploid ginger (*Zingiber officinale* Roscoe)

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Introduction

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

Profitability of the Australian ginger industry could be enhanced by improvement of cv. Queensland which has unique properties favoured during factory processing. Rhizomes bearing large knobs are preferred for processing into high quality confectionary ginger. Because the knob size is variable, and small knobs can not be satisfactorily used for 1st-grade confectionary ginger, rhizomes with small knobs are processed for less profit. Approximately 70% of the harvested ginger must be used for less profitable operations.

One technique to increase rhizome knob size is to induce autopolyploids as in some plant species a reasonable increase in numbers of genomes is accompanied by an increase in cell size and larger organs (Welsh 1981). Hamill *et al.* (1992) successfully developed a technique for producing banana autotetraploids by exposing micropropagated diploids to colchicine. This approach was followed for ginger (2n = 22). The results of the colchicine treatment and selection of tetraploid ginger are reported, together with evaluation of the first generation *ex vitro* plants in the field.

Materials and methods

Seed pieces of ginger (*Zingiber officinale* Roscoe) cv. Queensland were supplied by Buderim Ginger Ltd. and cultures were established after the method of Smith and Hamill (1996). Micropropagated ginger was capable of good multiplication rates, x4-5 per month, and sufficient material was available for colchicine treatment 12 months after initiation. The procedure for *in vitro* induction of autotetraploids was after the method of Hamill *et al.* (1992) and involved immersing shoot tips in a 0.5% w/v colchicine solution with 2% v/v DMSO for 2h under aseptic conditions.

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. 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 72h. Roots were rinsed in water and softened in 5N HCl for 25 min at room temperature then squashed and stained with an altered form of carbol fuchsin (Martens and Reisch 1988).

Colchicine-treated plantlets were subcultured over a 8 month period before being deflasked and acclimatised in a glasshouse. After 7 weeks the plants were taken to the Australian Golden Ginger Experimental Farm near Kandanga (26°10'S) and established in the field under 50% shade. The procedures for deflasking, field establishment and harvesting have been described previously (Smith and Hamill 1996).

Results and discussion

Colchicine Treatment

Explants treated with colchicine were initially slower growing than untreated control plants and mortality increased from 3% for the controls to 89% for the colchicine-treated plants after 8 weeks' culture. This compared to a 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. From subsequent chromosome counts it was 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.

Stomata Measurements for Determining Ploidy

The use of stomata measurements for screening plant populations treated with colchicine to select putative polyploids is well established (North 1979). Stomatal size differed significantly between the micropropagated diploid and tetraploid ginger (Figure 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 stomatal length ranged from 29.1-48.6 μm with a mean of 38.8 μm ($P<0.01$). There was no variation in stomatal length between upper or lower leaf surfaces nor was there variation between diploid plants from different culture bottles.

Stomatal measurements from field grown ginger showed similar trends to the *in vitro* material. The mean stomatal length of the autotetraploid was 50.8 μm compared to 36.7 μm for the diploid. There was no overlap in ranges of stomatal length. This data confirms the value of using stomatal impressions to detect changes in cell size and therefore more confidently select putative tetraploids from a colchicine-treated population.

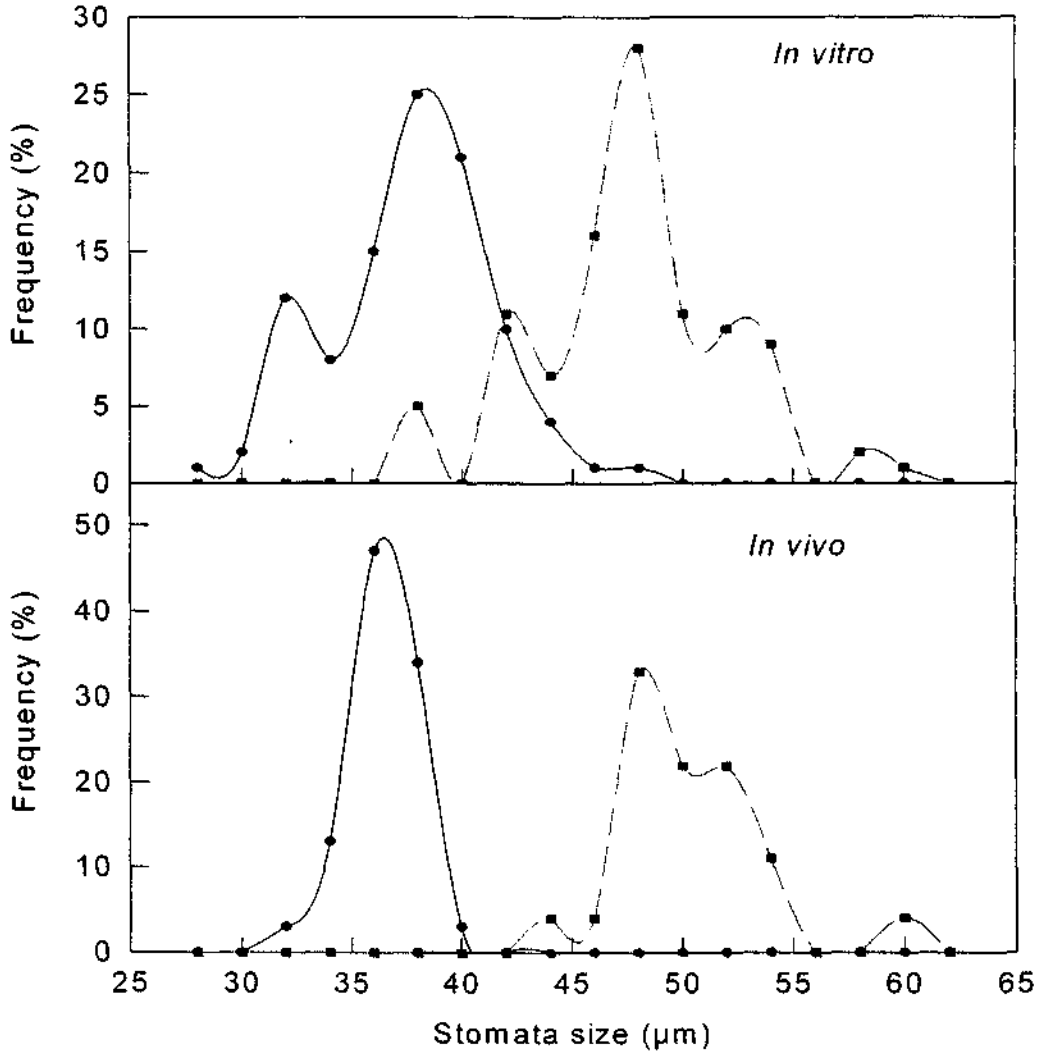
Stability of Colchicine-Treated Plants

The 15 autotetraploid plants selected in culture were micropropagated for a further 4 subculture cycles with a cytokinin, benzylaminopurine, in the culture medium to stimulate adventitious budding. The stimulation of adventitious buds following colchicine treatment has been shown to increase the percentage of solid, non-cyto-chimeral polyploids obtained in vegetatively propagated crops (Broertjes and van Harten 1988). Nevertheless, 3 of our ginger autotetraploid lines have reverted to the diploid state and one line has persisted with variegated leaves. Hamill *et al*'s (1992) work with banana autotetraploids also revealed reversions to the diploid level during micropropagation and even after field establishment. The possible reversion of autotetraploid ginger from field propagated material will have to be carefully monitored as it is increased for further field evaluation.

Evaluation of First Generation Ex Vitro *Autotetraploid Ginger*

The micropropagated ginger was planted on 20 October and the early harvest took place on 5 April when the flower heads emerged. This corresponded with a period of maximum recovery of 'choice' grade ginger. Choice grade ginger (used for confectionary) is when 35-45% by weight of the rhizome is free from commercial fibre ('fibre-free') (Whiley 1974).

Figure 1. Stomata size distribution of diploid and autotetraploid ginger. A minimum of 10 random stomatal measurements was taken for each of 24 diploid and 31 autotetraploid plants.



The contrasts between the micropropagated diploid and autotetraploid ginger was striking (Table 1). The autotetraploid had fewer but larger stems and leaves, and was similar in many respects to the diploid ginger propagated from conventional seed-pieces (sections of the rhizome treated with benomyl). The situation below ground also revealed similar differences.

The autotetraploid produced a larger rhizome and, although the number of knobs was similar to the micropropagated diploid, the average knob size was greater for the autotetraploid ginger. The rhizome of the micropropagated autotetraploid ginger was similar to the conventionally propagated diploid except for the greater mass of roots which seems to be a characteristic of the first generation of plants *ex vitro* (Smith and Hamill 1996).

Table 1. Shoot and rhizome characteristics of first generation *ex vitro* micropropagated and seed-derived ginger plants at early harvest. Plants were derived from diploid (Q2) seed or micropropagated (T.C.) diploid (Q2) or autotetraploid (Q4) plantlets. Values are means of 8 replicates. Means in rows followed by the same letters are not significantly different.

Characteristic	Seed (Q2)	T.C. (Q2)	T.C. (Q4)	l.s.d.	
				($P = 0.01$)	($P = 0.05$)
No. of shoots	9.3 a	27.0 b	14.5 a,b		12.6
Mean shoot length (cm)	86.6 a	57.2 c	70.8 b	20.2	14.1
Mean shoot mass (g_{fw})	62.3 a	18.8 b	52.5 a	19.5	
Mean leaf area (cm^2)	51.7 a	28.7 b	53.8 a	12.9	
Leaf area per shoot (cm^2)	846.3 a	416.5 c	677.4 b	203.6	139.9
Plant leaf area (cm^2)	9,992 a	11,288 a	9,818 a		
Mean stem mass (g_{fw})	43.1 a	17.6 b	42.8 a	11.8	
Mean leaf mass (g_{fw})	17.6 a	8.0 b	17.6 a	4.7	
Rhizome mass (g_{fw})	635.5 a	175.3 b	506.3 a	444.7	309.6
Root mass (g_{fw})	15.0 a	67.8 a	107.8 a		
Percentage of roots	2.4 a	30.5 b	17.6 a,b	27.3	
No. of knobs	58.0 a	49.8 a	57.5 a		
Mean knob mass (g_{fw})	10.9 a	3.4 b	8.9 a	4.4	

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 in all respects. This is despite the fact that the size of seed-pieces recovered from the micropropagated ginger's first generation crop is generally smaller. If the differences between the diploid and autopolyploid ginger continue into the second generation's crop then we can expect to see an improvement in recovery of rhizomes with larger knobs.

Plant material from the autotetraploid selections will be multiplied for further evaluation in the field and in the factory. To remain competitive and profitable, continued effort must be made in improving yield and recovery of premium-grade ginger.

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