Journal of Science and Technology in the Tropics (2010) **6**: 11-16

Effect of colchicine on tissue culture derived plants of *Zingiber officinale* Rosc. and *Zingiber officinale* var. *rubrum* Theilade

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Received 04-01-2010; accepted 01-02-2010

Abstract The aim of this study was to assess the effect of colchicine on the morphology and histology of gingers in view of polyploidisation. Rhizomes of ginger were treated separately with three concentrations of colchicine (0.5%, 1.0% and 2.0% w/v) for 30, 60 and 120 minutes. For morphology study, the fresh weight of rhizome (FWRH), length of root (RLEN) and plant height (PH) were measured. Histological analysis was done on shoot buds and rhizomes. All the parameters tested gave significant result. An increasing trend was observed with colchicine concentration up to 1.0%, but decreased at 2.0%. Histological analysis showed that the cell size of the shoot tip of *Zingiber officinale* Rosc. treated with colchicine (2.0%) for 120 minutes was 1.5 times larger than the control. For *Zingiber officinale* var. *rubrum* Theilade, the cell size was similar for treated and control shoot tips. Over all this study showed that polyploidisation might occur with colchicine treatment of various concentration and incubation time.

Keywords plant morphology – histology – polyploidisation – ginger

INTRODUCTION

Ginger, *Zingiber officinale* Rosc (Zingiberaceae), has been used as a spice both in the East as well as in the West since time immemorial [1]. In Peninsular Malaysia there are at least three local races, namely *halia betul* (true ginger), *halia bara* (red ginger) or alternatively *halia padi*, and *halia udang* [2]. Both *halia bara* and *halia padi* are distinguished from *halia betul* from their small rhizomes. *Halia udang* is probably extinct but *halia bara* differs slightly from *halia padi* by its externally red rhizome. *Halia bara* and *halia padi* are more pungent than the normal ginger and are mainly used in traditional medicine [2,3]. Pharmacological studies have shown that ginger rhizomes are effective for the recovery of intestinal disorder [4] and salivary secretion [5], for stimulating the vasomotor and respiratory centres, and for lowering serum and hepatic cholesterol levels [6,7].

Apart from the normal mitotic processes, polyploidy can be induced by treatment with colchicine [8-10]. The *in vitro* induction of polyploids with colchicine has been reported in many plant species [11-15]. The increase in the ploidy level could occur relatively easily and may lead to an overall enlargement of plant organs, for example cells, stomata, leaves, flowers, fruits and seeds [8].

Tetraploid gingers (*Z. officinale*) strains "4x Kintoki", "4x Sanshu" and "4x Phillippine Cebu 1" have been produced by soaking shoot tip explants in a colchicine solution (0.2%, w/v). The induced tetraploid gingers were much bigger in plant and rhizome size than the diploids [14]. In addition, the tetraploid ginger had higher pollen fertility and germination rates than the diploids. However, seed settings in the tetraploid ginger have not yet been reported [16].

Colchicine prevents spindle formation at prophase, precludes a nuclear mitosis, delays chromosomal separation, inhibits daughter nuclei, and effectively blocks the cleavage processes. Hence, when root tips

or other growing plant parts were placed in appropriate concentration of colchicine, the chromosomes of the treated cells duplicated without spindle formation and the cytoplasmic phase of cell division would not occur [17].

In general, colchicine is used as an antimitotic agent in plants. It influences the duplication of the number of chromosome. The effect of colchicine could be determined through the plant morphology. The present study was carried out to examine the effect of colchicine on the morphology and histology on tissue culture derived ginger plants *Zingiber officinale* and *Zingiber officinale* var. *rubrum*.

MATERIALS AND METHODS

Plant Material

The rhizomes of *Z. officinale* and *Z. officinale* var. *rubrum* used in this study were obtained from the local wet market. The specimens were authenticated by Halijah Ibrahim from the Institute of Biological Sciences, University of Malaya.

Application of colchicine

Colchicine solutions were cooled after autoclaving and were freshly prepared before use. Fourteen to sixteen shoot bud pieces were used for each treatment combination (Table 1). These buds were soaked in sterile distilled water (control), 0.5% (w/v), 1.0% (w/v) or 2.0% (w/v) aqueous colchicine solution in a 250-mL Erlenmeyer flask placed on an orbital shaker (90 rpm) at 24±2°C. The shoot buds were incubated according to the selected time (Table 1). Following that, each bud was dried on sterile filter paper and subsequently placed in MS semisolid initiation media under a 16 hours photoperiod at 3500 lx using white fluorescent tubes. The temperature was maintained at 24±2°C in the growth room. After about five months (5th subculture), the surviving colchicine-treated plantlets were transferred to sterile vermiculite placed in plastic polybags (3″ x 6″) and kept in a growth room under a 16:8-h light:dark photoperiod at 24±2°C for 4 months before transferring to the nursery.

Table 1. Combinations of treatment with colchicines concentration (C) and incubation time (T) used for *in vitro* derived shoot buds of ginger.

Concentration of Colchicines (%)	Incubation time (minutes)	Number of Shoot bud
CO (0.0)	TO (0)	16
	T1 (30)	16
	T2 (60)	16
	T3 (120)	16
C1 (0.5)	T0 (0)	14
	T1 (30)	14
	T2 (60)	14
	T3 (120)	14
C2 (1.0)	T0 (0)	14
	T1 (30)	14
	T2 (60)	14
	T3 (120)	14
C3 (2.0)	T0 (0)	14
	T1 (30)	14
	T2 (60)	14
	T3 (120)	14

Growth maintenance of colchicine treated plants

After the plantlets had rooted and reached 3-5 cm in height with well expanded leaves, they were transplanted into pots filled with soil mix consisting of sand:peat:top soil (3:2:1), vermiculite and fertilizer (N:P:K at the ratio of 15:15:15) after washing off the agar with tap water. To avoid fungal infection, the soil mix in the pot was drained with 1% (w/v) Thiram® over night before planting. For pest control, plants were sprayed with 1.0 mL of Plusbon 250® diluted with 5 L water. Plants were irrigated daily and 1.0 g of foliar fertilizer (Agrospray 63®) diluted in 5 L tap water was sprayed monthly for supplement.

Measurement of plant morphology

The plants were monitored every month. They were maintained in the nursery for one year before harvesting and analysis. For each treatment, fresh weight of rhizome (FWRH) and plant height (PH) were measured. Length of the root (RLEN) was measured randomly by using Image-Pro® Express Version 4.5 (Media Cybernetics).

Histological analysis

The shoot buds were fixed in FAA (formalin, acetic acid glacial, ethyl alcohol: 5:5:90), then progressively dehydrated in ethanol series, and finally infiltrated in paraffin. Longitudinal sections (10-20 µm thick) of paraffin-embedded materials were obtained using a

rotary microtome. The sections were stained with a mixture of 1% (v/v) safranine and 1% (v/v) fast green and mounted with Canada Balsam [18]. Size of cells was measured using a micrometer eyepiece where 0.01 mm equals to 5 mm (under x100 and x400 magnification). This work was done at the laboratory of plant histology, Biological Faculty, University of Gadjah Mada, Yogyakarta, Indonesia.

Statistical analysis

Analysis of variance (ANOVA), performed with the program SAS (SAS® proprietary software Release 6.02), of factorial design was used to test the effect of the concentrations of colchicine and incubation times. Duncan Multiple Range Test (DMRT) at $p<0.05$ was used to test for differences between the control and each of the treatment [19].

RESULTS AND DISCUSSION

Morphology of *ex vitro* derived treated plants during Hardening

In the nursery, plants that were unaffected by the treatments survived and grew vigorously as the control plants. No abnormal plants were found during hardening (Fig. 1). However, a few plants failed to survive in some concentrations of colchicine.

Treatment with increasing colchicine concentration produced decreasing measurement of the fresh weight of rhizome, plant height and root length (Table 2). For incubation time, T1 (30 minutes) produced higher measurement values compared to control ($p<0.05$). It can be concluded that T1 is the best incubation time for this experiment.

Based on the results (Table 3), the fresh weight of rhizome (FWRH) for *Zingiber officinale* did not show overlapping values from each concentration of colchicine and incubation time. The measurement values were higher at colchicine concentration of 2.0% (C3) and incubation time 60 minutes (T2), compared to colchicine concentration of 0.5% (C1) and incubation time 30 minutes (T1). For *Zingiber officinale* var. *rubrum* the rhizome weight was heavier at colchicine 1.0% (C2) and incubation time 30 minutes (T1) than colchicine 0.5% (C1) and 30 minutes incubation (T1) (Fig. 2).

Table 2. Fresh weight (g) of rhizome (FWRH), plant height (PH, cm) and length of root (RLEN, cm) of ginger after transferring to the nursery. Different alphabets show significant differences ($p<0.05$), based on Duncan's Multiple Range Test (DMRT). C, concentration of colchicine; T, incubation time.

Parameter	Treatment							
	C0	C1	C2	C3	T0	T1	T2	T3
FWRH	64.90a	42.36b	28.39c	23.52d	50.79b	55.24a	21.41d	31.72c
PH	35.67a	26.46b	14.30c	9.87d	24.46b	31.03a	9.82d	20.99c
RLEN	18.38a	13.60b	7.85c	3.75d	13.58b	15.91a	4.85d	9.23c

Figure 1. Vigorous growth of treated plants (C1T1) – *Zingiber officinale* (left) and *Zingiber officinale* var. *rubrum* (right) – after transferring to the nursery.

Plant height measured for *Z. officinale* (V1) showed the highest reading (48.24 cm) for treatment C1T1 compared to the other treatments. C1T1 treatment for *Z. officinale* var. *rubrum* (V2) exhibited similar result for the control (43.38 cm) and treated group (43.16 cm) (Table 3.). Roots for *Z. officinale* (V1) were shorter compared to *Z. officinale* var. *rubrum* (V2). The best result for *Z. officinale* was produced by treatment C1T1 while for *Z. officinale* var. *rubrum* the longest root measurement was obtained when the shoot bud was briefly dipped in treatment C1T0.

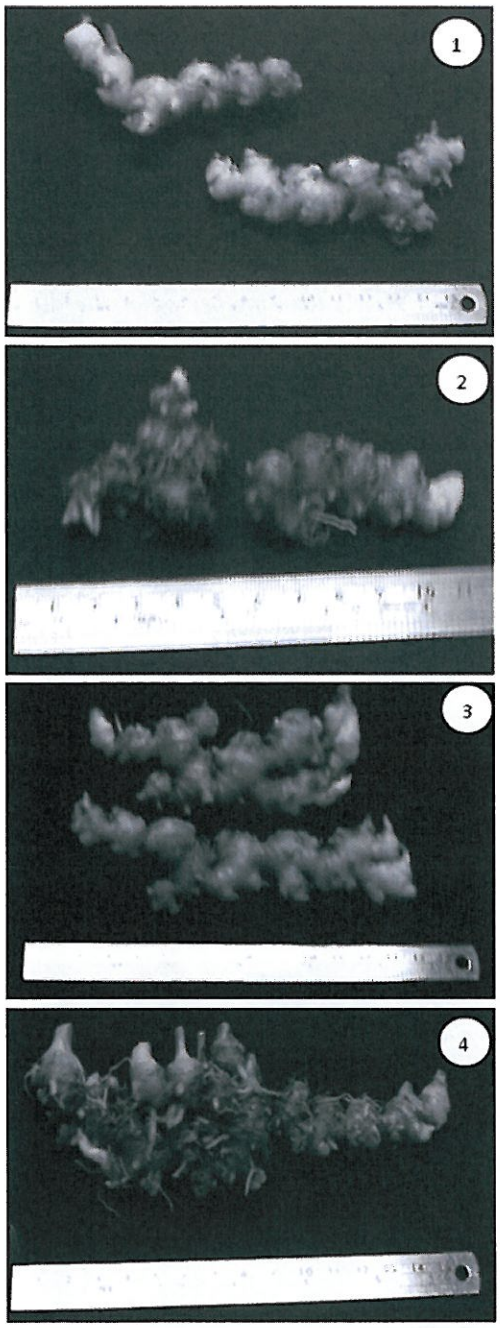

Figure 2. Rhizome of *Zingiber officinale*, V1, C0T0 (1) and *Zingiber officinale* var. *rubrum*, V2, C0T0 (2) obtained from control; and rhizome of *Zingiber officinale*, V1, C3T2 (3) and *Zingiber officinale* var. *rubrum*, V2, C1T1 (4) after treatment. C0T0 = Control; C3T2 = colchicine conc. 2%, incubation time 60 min; C1T1 = Colchicine conc. 0.5%, incubation time 30 min.

To date, there are no reports on the morphology of *ex vitro* colchicine treated plants as described at this study. Based on this study, an increasing trend for fresh

Table 3. Fresh weight of rhizome (FWRH, g), PH, plant height (PH, cm) and root length (RLEN, cm) from combination of variety, concentration of colchicine and incubation time after transferring to the nursery. V, Variety (V1, *Zingiber officinale*; V2, *Zingiber officinale*. var. *rubrum*); C, concentration of colchicine; T, incubation time. Different alphabets show significant differences ($p<0.05$), based on Duncan's Multiple Range Test (DMRT).

No.	Combination of variety, concentration of colchicine and incubation time	DMRT grouping		
		FWRH	PH	RLEN
1	V1C0T0	141.47a	45.46b	15.45k
2	V1C0T1	81.73f	42.89cd	22.46f
3	V1C0T2	0.00p	0.00k	0.00n
4	V1C0T3	87.05e	41.26ef	19.22hi
5	V1C1T0	80.96f	42.50cd	14.84lm
6	V1C1T1	49.47m	48.24a	19.56h
7	V1C1T2	0.00p	0.00k	0.00n
8	V1C1T3	61.68j	42.07de	19.25hi
9	V1C2T0	0.00p	0.00k	0.00n
10	V1C2T1	58.97k	36.35i	17.35j
11	V1C2T2	0.00p	0.00k	0.00n
12	V1C2T3	0.00p	0.00k	0.00n
13	V1C3T0	0.00p	0.00k	0.00n
14	V1C3T1	0.00p	0.00k	0.00n
15	V1C3T2	120.13b	37.72h	15.28kl
16	V1C3T3	68.05i	41.21ef	14.74m
17	V2C0T0	30.97o	32.73j	22.54f
18	V2C0T1	89.79d	38.80g	23.14e
19	V2C0T2	51.18l	40.85f	23.59d
20	V2C0T3	37.01n	43.38c	20.67g
21	V2C1T0	76.50g	35.72i	29.32a
22	V2C1T1	70.25h	43.16c	25.81c
23	V2C1T2	0.00p	0.00k	0.00n
24	V2C1T3	0.00p	0.00k	0.00n
25	V2C2T0	76.43g	39.25g	26.47b
26	V2C2T1	91.71c	38.78g	18.98i
27	V2C2T2	0.00p	0.00k	0.00n
28	V2C2T3	0.00p	0.00k	0.00n
29	V2C3T0	0.00p	0.00k	0.00n
30	V2C3T1	0.00p	0.00k	0.00n
31	V2C3T2	0.00p	0.00k	0.00n
32	V2C3T3	0.00p	0.00k	0.00n

weight of rhizome, plant height and root length was observed with increasing colchicine concentration up to 1.0% but decreased at 2.0%. For incubation time, results were not consistent. Based on morphological characteristics (fresh weight of rhizome, plant height and root length) for treated plants, significant results were found in all the data. When colchicines was used, various organs of tetraploid gingers become huge in appearance i.e. plant height, the size of leaves and rhizomes, compared to diploids [14].

Histological analysis of shoot tips

Preliminary results showed that the cell size of the shoot tip treated with colchicine (2.0%, C3) and incubation time (T3, 120 minutes) was 1.5 times larger than the control (C0) for sample V1 (Fig. 3). For sample V2, the treated and control shoot tips had similar cell size (Fig. 4).

From this preliminary histological analysis, the effects of colchicine concentration were observed for sample V1, but the result obtained did not confirm or prove that polyploidisation was induced by the treatments. Future work on quantitative analysis is needed.

CONCLUSION

The effect of colchicine treatment on *Z. officinale* and *Z. officinale* var. *rubrum* could be observed in the morphology and histology of the treated plants. An increase in the fresh weight of the rhizomes of the treated plants was observed as compared to the control. However, the results from qualitative histological studies were inconclusive.

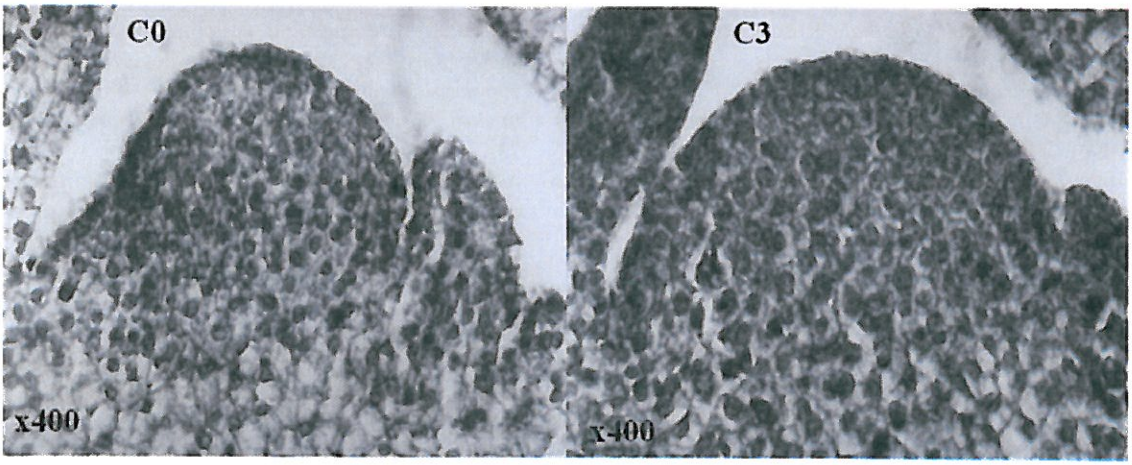

Figure 3. Micrographs of a section of the shoot tips of *Zingiber officinale* (V1). C0 (control) and C3 (treatment with colchicine concentration 2.0% and incubation time 120 minutes).

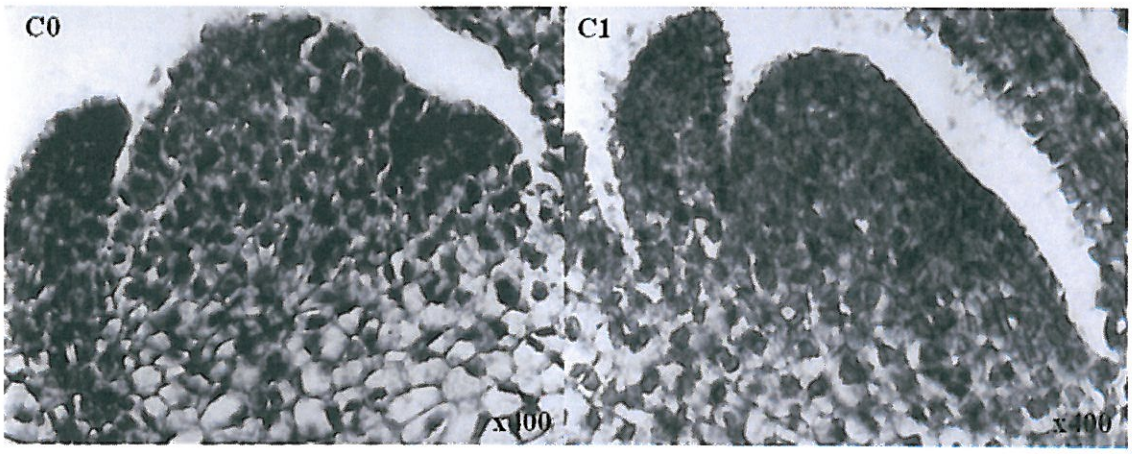

Figure 4. Micrographs of a section of the shoot tips of *Zingiber officinale* var. *rubrum* (V2). C0 (control) and C1 (treatment with colchicine concentration 0.5% and incubation time 30 minutes).

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