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In Vitro Propagation of Ginger (*Zingiber officinale* Roscoe)

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ABSTRACT

Disease free ginger plantlets were produced through tissue culture. The method for surface sterilization was soaking the emerging buds in distilled water containing 0.2% Tetracycline and 0.2% Metalaxyl for one and half hour before treated with 10% clorox for 15 minutes followed by 5% clorox for 10 minutes. Shoot tips were excised from the buds and planted onto MS medium supplemented with 3 mg/l BA. Eighteen percent of the explants were obtained as clean culture. The uncontaminated shoot tips were transferred to MS medium supplemented with 5 mg/l BA for shoot growth. Shoot tips were then cultured on MS and 2 g/l Twin Forty (a commercial fertilizer medium containing N, P_2O_5 and K_2O 30:20:10 respectively) media with and without supplementing plant growth regulators. The supplemented plant growth regulators were 5 mg/l 6-benzylaminopurine (BA) in combination with 0.5 mg/l naphthaleneacetic acid (NAA) and 4 ml/l Surprise (a concentrated liquid plant food which contains natural biopolymer with organic acid and inert substances). The purpose of the study was to sort out the simple and cost effective medium and plant growth regulator for *in vitro* propagation of ginger with high multiplication rate. All the cultures were incubated at 25±2°C and 12 hours photoperiod. Pseudostems cultured on MS basal medium supplemented with 5 mg/l BA in combination with 0.5 mg/l NAA produced the highest number of shoots with an average of 5.33 shoots per pseudostem after 5 weeks of culture. Similarly length of shoots was highest on the same medium. Twin Forty fertilizer medium without supplementing with any plant growth regulators produced the lowest number of shoots, leaves and length of shoots. The cost of materials per plantlet was lowest on MS and Twin Forty fertilizer media supplemented with BA in combination with NAA. The results revealed that MS and Twin Forty media supplemented with plant growth regulators such as BA in combination with NAA are more effective than Surprise and without supplementing any plant growth regulators for cost effectiveness and rapid multiplication.

Key words: tissue culture, propagation, *Zingiber officinale*, ginger

INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) is a herbaceous perennial commercially grown as an annual which has long been used as spice and medicine in Asia. Since flowering is rare and no viable seeds are produced, it is generally propagated from matured rhizome seed pieces having three to four buds (Ikeda and Tanabe, 1989; Malamug *et al.*, 1991). Conventional multiplication technique

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through seed rhizomes produces only 10-15 lateral buds in a season of 8-10 months (Bhagyalakshmi and Singh, 1988). In addition, this crop is heavily attacked by bacterial wilt (*Pseudomonas solanacearum*), fusarium yellows (*Fusarium oxysporum f. zingiberi*) and root knot nematode (*Meloidogyne incognita*). Fusarium yellows in the field is positively correlated with its occurrence in storage and 87% of the pre-emergence rot and yellows is transmitted from infected rhizomes (Dohroo, 1989). Three-fold increase in production is possible with effective disease control (Hosoki and Sagawa, 1977). It is therefore necessary to produce disease free seed rhizomes or clones with rapid multiplication rate.

Millions of plantlets can be produced within 1-2 years from a single explant and tissue of ginger has been cultured *in vitro* and consequently freed of pathogens (Sahavacharin, 1995). Bacteria, fungi, viruses and nematodes are successfully eliminated from infected plants through *in vitro* culture of shoot tips (De Lange *et al*, 1987; Sahavacharin, 1995). Many workers have reported rapid clonal propagation of ginger on MS medium with varying concentration of plant growth regulators such as BA and NAA. Though micropropagation has been one of the most important ways to produce crops which are difficult to propagate by conventional methods, this technology has commonly been used in high value, low volume cash crops. This method still requires high cost of multiplication of disease free plantlets in the laboratory. One of the alternative strategy for cost reduction involves the use of cheaper media and plant growth regulators with high multiplication rate. The objective of this study was to analyze the rapid multiplication of plantlets using the cost effective medium and plant growth regulators.

MATERIALS AND METHODS

Rhizomes of ginger (*Zingiber officinale* Roscoe), cultivar King Yai were used in the experiment. New emerging buds (about 2 cm) from the rhizomes (Figure 1 A) were cut and washed in running water for 5 minutes. The buds were rinsed with 70% ethyl alcohol and then soaked in sterilized water containing 0.2% Tetracycline and 0.2% Metalaxyl for one and half hour. The buds were then surface sterilized by submerging buds in 10% Clorox + 2 drops of Tween 20 for 15 minutes. The buds were again submerged in 5% Clorox + 2 drops of Tween 20 for 10 minutes. The buds were finally rinsed three times with sterilized water.

Shoot tip explants trimmed to 2-3 mm height were excised from the buds and planted onto MS medium (Figure 1 B) supplemented with 3 mg/l BA and 3% sucrose, 0.62% agar as the solidifying agent. The pH of the medium was adjusted to 5.6 with NaOH. Eighteen percent of the explants were obtained as clean culture and most of the explants contamination was caused by bacteria.

The uncontaminated shoot tips were then transferred to a fresh MS medium supplemented with 5 mg/l BA (Figure 1 C and D). The cultured condition was 25±2°C and 12 hours photoperiod.

Pseudostems of leafy aerial shoots consisting mainly of tightly sheathed leaf bases (Figure 1 D) were selected from plantlets produced *in vitro* and used in the experiment. Cutting shoots averaged 15 mm in length were cultured on Murashige and Skoog (1962) basal medium and 2 g/l Twin Forty (a fertilizer medium containing N, P_2O_5 and K_2O 30:20:10 respectively). Both the media were used with and without supplementing plant growth regulators. The supplemented plant growth regulators as reported by Inden *et al.* (1988) were 5 mg/l 6-benzylaminopurine (BA) in combination with 0.5 mg/l naphthaleneacetic acid (NAA) and these were compared with 4 ml/l Surprise (a

concentrated liquid plant food which contains natural biopolymer with organic acid and inert substances). Sucrose of 3% and 0.62% agar were added and pH was adjusted to 5.6. Twenty five ml medium was dispensed into 75×40 mm size bottles with plastic caps and then sterilized at 121°C under 1.05 kg/cm2 pressure for 20 minutes. Three pseudostems were placed in a bottle and ten bottles per treatment were observed. After culturing, cultured media were incubated at 25±2°C and 12 hours photoperiod. The number of shoots, roots, leaves per pseudostem and length of shoots in the fifth week of culture were recorded and analyzed. The cost of basal media and supplements was calculated assuming the same labor and utilities cost in all the treatments.

RESULTS AND DISCUSSION

MS basal medium supplemented with 5 mg/l BA in combination with 0.5 mg/l NAA was the most effective in producing adventitious shoots from pseudostems. It produced the highest number of shoots with an average of 5.33 shoots per pseudostem followed by Twin Forty fertilizer medium (2 g/l) supplemented with 5 mg/l BA in

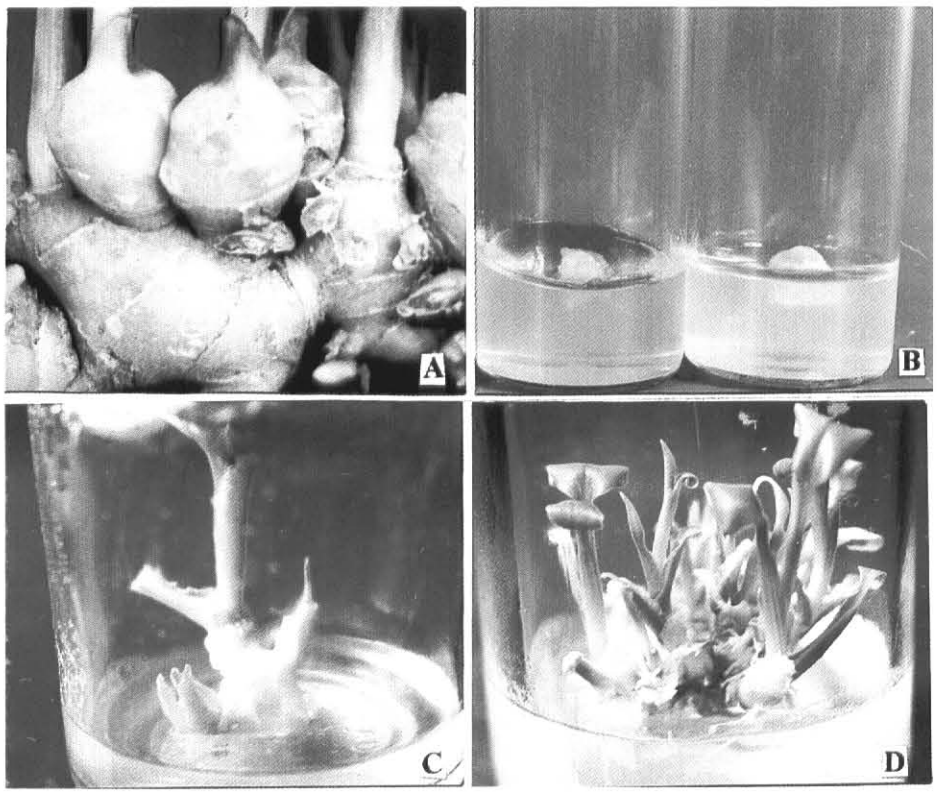

Figure 1 Establishment of an aseptic culture
(A) Ginger rhizomes with emerging buds
(B) Surface sterilized buds cultured on MS medium + 3 mg/l BA
(C) Shoot growth on MS medium + 5 mg/l BA
(D) Adventitious shoots on MS medium + 5 mg/l BA

combination with 0.5 mg/l NAA with an average of 3.53 shoots per pseudostem (Table 1 and Figure 2). Twin Forty fertilizer medium without supplementing plant growth regulators produced the lowest number of shoots per pseudostem with an average of 1.13 shoots per pseudostem. The effect of Surprise was not satisfactory on producing shoots as compared to BA in combination with NAA. MS and Twin Forty media supplemented with 4 ml/l Surprise produced an average of 1.60 and 1.37 shoots per pseudostem respectively.

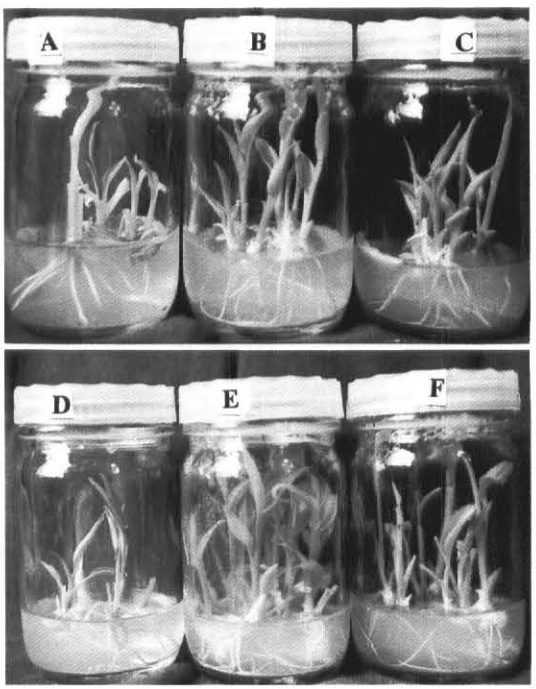

Figure 2 Shoot multiplication on different medium and plant growth regulators (after 5 weeks of culture)
(A) Twin Forty
(B) Twin Forty + BA + NAA
(C) Twin Forty + Surprise
(D) MS
(E) MS + BA + NAA
(F) MS + Surprise

Similarly, the difference among the treatments on length of shoots and average number of roots were found to be highly significant (Table 2). The length of shoots after 5 weeks of culture was highest on MS medium supplemented with BA in combination with NAA. And the average number of roots was highest on Twin Forty fertilizer medium supplemented with BA in combination with NAA. However, the average number of leaves per shoot was not significantly different in all the treatments except Twin Forty fertilizer medium without supplemented with growth regulators.

Cost of media and supplements was calculated assuming all other labor and utilities costs the same in all the treatments. The cost of materials per plantlet was lowest on MS and Twin Forty fertilizer media supplemented with BA in combination with NAA with an average of 0.05 Baht per plantlet and the highest cost was in MS medium without plant growth regulators with an average of 0.14 Baht per plantlet. However, the cost of materials was more in MS medium as compared to fertilizer medium (Table 1).

The results supported the findings of Ikeda and Tanabe (1989) that MS medium supplemented with 11 M BA in combination with 0.6 M NAA could produce 5 shoots per pseudostem. Inden *et al.* (1989) reported that more than 750,000 plants could be produced after one year from a single shoot tip on MS medium supplemented with 5 mg/ BA and 0.5 mg/l NAA. Balachandran *et al.* (1990) also produced 4.05 shoots per explant on MS medium supplemented with 3 mg/l BA. However, shoots/pseudostem were lowest on commercial fertilizer medium without supplementing plant growth regulators than any other reports.

Higher multiplication rate on MS medium than fertilizer medium might be due to the content of essential nutrients and vitamins. MS contains more essential nutrients and vitamins but commercial fertilizer medium is lacking of vitamins

Table 1 Effects of media and plant growth regulators on shoot multiplication and cost of ginger plantlets after 5 weeks of culture.

Media and plant growth regulators	Average no. of plantlets produced	Cost of materials (Baht/plantlet)
Twin Forty	1.13 d[1]	0.07
Twin Forty+BA+NAA	3.53 b	0.05
Twin Forty+Surprise	1.37 cd	0.07
MS	1.33 cd	0.14
MS +BA + NAA	5.33 a	0.05
MS +Surprise	1.60 c	0.13
CV %	12.6	
F - Test[2]	**	

1 Means followed by a common letter are not significantly different at the 5 % level by DMRT

2 ** = significant at p 0.01

Table 2 Effects of different media and plant growth regulators on *in vitro* shoot growth of ginger after 5 weeks of culture.

Media and plant growth regulators	Length of shoots (cm)	No.of roots/shoot	No.of leaves/shoot
Twin Forty	2.43 f[1]	4.20 c	2.57 b
Twin Forty+BA+NAA	4.62 b	7.33 a	3.23 a
Twin Forty+Surprise	2.79 e	4.37 c	3.12 a
MS	3.51 d	4.03 c	3.14 a
MS +BA + NAA	5.84 a	6.94 b	3.19 a
MS +Surprise	3.88 c	4.30 c	3.13 a
CV %	2.4	7.2	4.5
F - Test[2]	**	**	**

1/ Means followed by a common letter are not significantly different at the 5 % level by DMRT

2/ ** = significant at p 0.01

and balanced quantity of nutrients. Similarly, plant growth regulators such as BA and NAA are more effective for shoot and root differentiation and are widely used.

Since the cost of Twin Forty fertilizer medium was less than MS medium and the cost of Surprise was less than BA in combination with NAA, multiplication rate overrides the cost of materials. Cost of materials per plantlet could be reduced by more than 64% on MS medium supplemented with BA in combination with NAA as compared with those without supplements and those supplemented with Surprise. Similarly, the cost of materials per plantlet on Twin Forty fertilizer medium supplemented with BA in combination with NAA could be reduced by 28% as compared with those without supplements and those supplemented with Surprise. The result of this experiment suggests that high multiplication rate is more important than cost of materials for reducing cost of plantlets *in vitro*.

CONCLUSION

MS medium is better than Twin Forty fertilizer media for producing more and better shoots. Plant growth regulators such as BA in combination with NAA are more effective than Surprise (a concentrated liquid plant food) for producing more shoots and reducing cost of materials. MS and Twin Forty media supplemented with plant growth regulators such as BA in combination with NAA are more cost effective than without supplement.

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