

Micropropagation Study on Three Varieties of *Zingiber officinale* Rosc.

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Received 3 December 2002, accepted in revised form 30 August 2004

ABSTRACT The *in vitro* propagation protocols for three varieties of *Zingiber officinale* were successfully established in this study. Averages of three to five shoots per explant were successfully regenerated on MS medium supplemented with 3.0% (w/v) sucrose, 0.2% (w/v) phytigel, and 1.0-3.0 mg/l BAP. All varieties studied have shown that the rhizome yield from *in vitro* plantlets were higher than those from *in vivo* materials. The protocols developed can be adopted for propagation and conservation purposes.

ABSTRAK Protokol propagasi secara *in vitro* ke atas tiga varieti *Zingiber officinale* telah berjaya dilakukan di dalam kajian ini. Secara purata antara tiga hingga lima pucuk setiap eksplan berjaya diregenerasikan di dalam media MS yang mengandungi 3.0% (b/i) sukrosa, 0.2% (b/i) fitagel dan BAP pada kepekatan 1.0-3.0 mg/l. Kesemua varieti yang dikaji menunjukkan penghasilan rizom yang lebih baik daripada plantlet *in vitro* berbanding dengan *in vivo* semasa ditanam di lapangan. Dengan itu, kaedah kultur tisu yang telah dirumuskan dalam kajian ini, amat sesuai digunakan untuk tujuan propagasi dan pemuliharaan varieti-varieti yang dikaji.

(*Zingiber officinale*, micropropagation, shoot buds)

INTRODUCTION

Ginger (*Z. officinale* Rosc.) belonging to the family Zingiberaceae is an important spice crop and is also widely used in traditional medicine and culinary preparations. In practice, it is vegetatively propagated through underground rhizomes, but at a very low multiplication rate. Heavy losses in yield have been reported due to bacterial wilt (*Pseudomonas solanacearum*), soft rot (*Phytium aphanidermatum*) and nematodes (*Meloidogyne* spp.) [1, 2]. Since these diseases are mainly transmitted through rhizomes, the production of disease-free clones with a rapid multiplication rate is necessary for a successful commercial cultivation of this crop.

In the present study, experiments were conducted to establish micropropagation protocols from ginger shoot buds using various concentrations of growth hormone. Three varieties of *Z. officinale* with valuable medicinal properties were used as plant materials which are locally known as

'halia' (*Z. officinale* Rosc. var. *officinale*), 'halia bara' (*Z. officinale* Rosc. var. *rubrum* Theilade) and 'halia padi' (*Z. officinale* Rosc. var. *rubrum* Theilade).

MATERIALS AND METHODS

Four week-old shoot buds from the varieties studied were used as experimental materials. Shoot buds were washed thoroughly and the outer leaves were removed and subsequently explants were cut into 2-3 cm sections. Buds were soaked in 15.0% (v/v) domestic chlorox including 1-2 drops Tween 20 for 15-20 minutes followed by 0.5% (w/v) HgCl₂ for five minutes, 70% (v/v) ethanol (one minute) and then rinsed in sterile distilled water (three times). After sterilisation, buds were cut approximately to 1.0 cm and were placed into culture medium.

Murashige and Skoog (MS) [3] were used as the basal medium containing 3.0% (w/v) sucrose and 0.2% (w/v) phytigel. Different concentrations of

BAP (0-10.0 mg/l) were added to the basal medium. The pH of the medium was adjusted to 5.8 before autoclaving. The cultures were incubated at 25±2°C and 16 hours photoperiod.

Contaminant free explants grown on the MS medium without hormones for eight weeks were then transferred to fresh MS media supplemented with different concentrations of BAP for shoot multiplication. Repeated subcultures were carried out until four times at four week intervals before a suitable and optimum medium was determined. The number and length of shoots and roots obtained per explant were recorded for each subculture. Explants that produced the highest number of new shoots in the optimum medium were selected for further subcultures.

Plantlets with well developed shoots and roots were transferred to soil mixed with 'cocopeat' (1:1) for field evaluation. Micro propagated plants were compared with conventional propagated plants for various morphological and rhizome characters. The control plants were initially taken from one well rooted shoot, excluding the rhizome and were planted in uniform sized pots as the ones used for growing the *in vitro* plantlets. Data on plant height, number of tillers per plant, leaf area, number of leaves and rhizome weight were determined after 8 months of field transplantation.

RESULTS AND DISCUSSION

All varieties of *Z. officinale* best responded on MS medium with 3.0% (w/v) sucrose, 0.2% (w/v) phytigel supplemented with low concentrations of BAP (1.0-3.0 mg/l). Two to eight shoots per explant were produced with simultaneous root formation. Two types of shoots were initiated from one explant i.e single shoots from the main axis and axillary buds at the base of the explants.

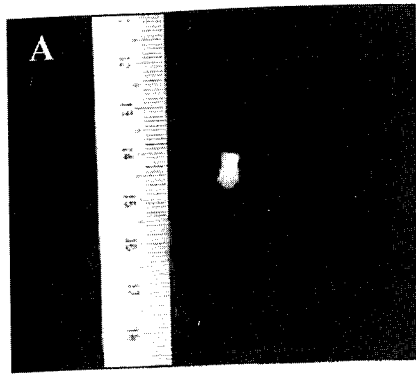
The optimum level of BAP is 1.0 mg/l for 'halia' and 'halia bara' and 3.0 mg/l for 'halia padi'. The optimum media were selected based on the highest number of shoots produced. At higher concentrations of BAP (5.0-10.0 mg/l), less number of shoots was formed.

Shoots produced were compact, folded and recalcitrant to elongation. Very rare or no rooting were observed for all varieties. This showed that propagation was only suitable at low concentrations of BAP for the varieties studied. Similar results were also reported by Sharma and Singh [5] where kinetin was used to propagate *Z. officinale*. At high concentrations of kinetin, less shoot elongation, folded leaves and very rare rooting were observed. Upadhyay *et al.* [6] made an in-depth study on the influence of cytokinins (BA, 2ip and kinetin) individually where each cytokinin was used at high concentrations (3.0-5.0 mg/l) in the media. It was observed that an increase in the concentration led to an increase in the frequency of the incidence of abnormalities such as vitrification and fasciation of the shoots formed.

After three subcultures (4 weeks intervals) on optimum medium, no adverse effect was observed either on the rate of proliferation or quality of plantlet. Table 1 showed that 'halia padi' was more productive in terms of shoot multiplication compared to other varieties cultured in the optimum media.

In vitro derived plantlets with well-developed root and shoot system were successfully transferred to pots in potting mixture containing soil and cocopeat (1:1), and healthy rhizomes were obtained after harvesting. The performance of micro propagated plants for five quantitative traits under field conditions is given in Table 2. Average plant height, number of tillers per plant, number of leaves, leaf area and rhizome weight per plant for all varieties revealed that the micro propagated plants were superior compared to the control plants. The higher number of tillers and leaves suggested a residual effect of the cytokinins used in the culture medium [4].

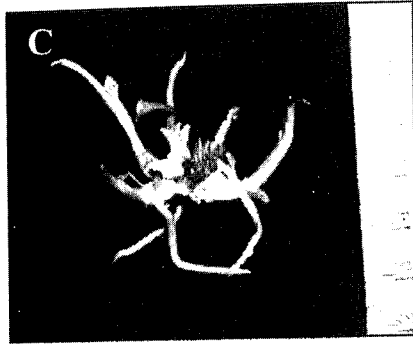
The present experiment has demonstrated a very simple protocol for the rapid propagation of a medicinally important herb such as *Z. officinale*. This protocol may also be adapted as a part of the *in vitro* conservation for germplasm collection.



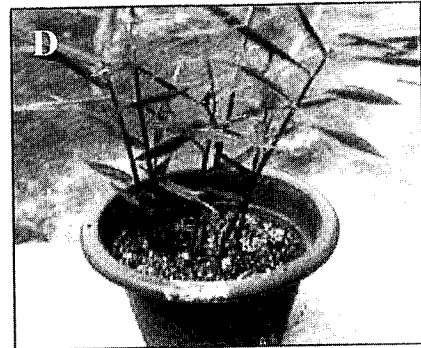
Explant



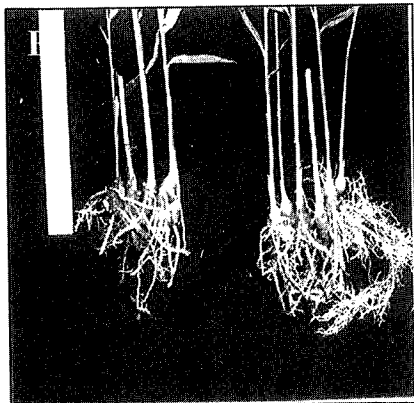
Initiation stage
(Establishment of contaminant-free explant)



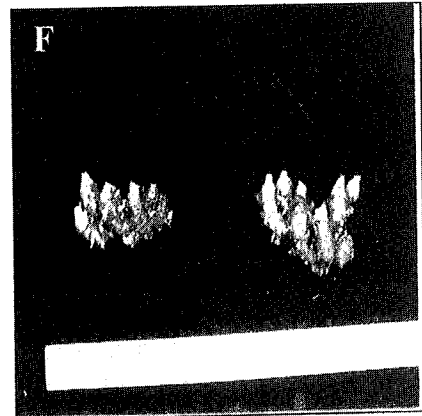
Shoot multiplication (2 weeks in culture)



Field planting



in vivo plant (left) and *in vitro* plant (right)



in vivo rhizome (left) and *in vitro* rhizome (right)

Figure 1. Stages of micro propagation on '*Halia barua*'

Table 1. Number, average number and maximum length of shoots and roots per explant produced on optimum medium after 3 subcultures.

| Species | Optimum medium | Number of shoots per explant | Average number of shoots per explant | Number of roots per explant | Average number of roots per explant | Max. length of shoot (cm) | Max. length of root (cm) |
|-------------------|-------------------|------------------------------|--------------------------------------|-----------------------------|-------------------------------------|---------------------------|--------------------------|
| <i>Halia</i> | MS + 1.0 mg/l BAP | 2-6 | 4.2 ± 0.2 | 7-18 | 11.5 ± 0.3 | 5.0 | 7.5 |
| <i>Halia bara</i> | MS + 1.0 mg/l BAP | 2-5 | 3.3 ± 0.2 | 5-17 | 9.9 ± 0.4 | 4.5 | 7.0 |
| <i>Halia padi</i> | MS + 3.0 mg/l BAP | 3-8 | 4.8 ± 0.5 | 8-17 | 11.8 ± 0.9 | 4.5 | 8.0 |

Table 2. Field performance of micro propagated and control plants for five quantitative traits

| Parameter study | <i>Halia</i> | | <i>Halia bara</i> | | <i>Halia padi</i> | |
|------------------------------|-----------------|-------------|-------------------|------------|-------------------|------------|
| | <i>in vitro</i> | control | <i>in vitro</i> | control | <i>in vitro</i> | control |
| No. of tillers | 6.0 ± 1.0 | 4.6 ± 0.9 | 4.8 ± 0.8 | 3.4 ± 0.6 | 6.6 ± 0.6 | 4.4 ± 1.1 |
| Plant height (cm) | 56.3 ± 5.2 | 57.2 ± 9.6 | 26.3 ± 2.3 | 24.5 ± 3.9 | 24.3 ± 6.1 | 24.8 ± 4.7 |
| No. of leaves | 64.8 ± 16.3 | 54.8 ± 7.4 | 35.0 ± 6.4 | 31.4 ± 3.1 | 52.0 ± 15.8 | 33.0 ± 4.7 |
| Leaf area (cm ²) | 27.6 ± 1.2 | 25.9 ± 1.4 | 20.5 ± 0.4 | 18.2 ± 0.6 | 17.4 ± 0.7 | 17.2 ± 0.3 |
| Fresh weight of rhizome (g) | 50.7 ± 23.3 | 38.4 ± 20.0 | 19.3 ± 3.2 | 12.0 ± 5.0 | 21.8 ± 4.5 | 11.3 ± 4.9 |

Acknowledgements We wish to thank the Ministry of Science, Technology and Environment (NSF scholarship and IRPA grant: 08-02-03-0229.) and University of Malaya (Vote F (2000&2001) for the financial support.

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