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Micropropagation of red ginger (*Zingiber montanum* Koenig), a medicinal plant

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Abstract. A protocol has been developed for the in vitro regeneration of red ginger (Zingiber montanum Koenig), a valuable medicinal plant. Rhizome buds of 1.5 to 2 cm long were surface sterilized with commercial bleach prior to culture on Gamborg B5 medium incorporated with Tetracycline at 15 ml/L and Plant Preservative Mixture at 2 ml/L. For induction of multiple shoots, three studies were conducted, (1) effects of different plant growth regulators, (2) effect sof whole and sectioned buds and (3) effects of culture phase. Thidiazuron (TDZ) at 0.5 mg/L was found to induce the highest shoot multiplication with a mean of 8.1 shoots per explant. Sectioned buds produced a mean of 4.6 shoots from each explant. As far the culture phase, liquid medium was found to be superior to solid medium. Rooting of propagules was conducted on B5 medium devoid of growth regulators. Acclimatization was conducted on medium containing a mixture of 1:1:1 soil, sand and peat with about 85% survivability.

Keywords: *In vitro* regeneration; Whole buds; Sectioned buds; Culture phase.

INTRODUCTION

Zingiber montanum Koenig syn. *Zingiber cassumunar* Roxb. belongs to the family *Zingiberaceae*. The species is native to India. It is known as bonglai in peninsular Malaysia, bangle in Java and plai in Thai. There are two forms of *Z. montanum*, one with yellow rhizome skin but creamy white flesh and another with red skin but yellow flesh. In this study, the red skin type was chosen. It has a pungent odour and a foul-smelling flower (Boyce, 2006). This plant is highly valued for its medicinal properties. In Malaysia, the rhizome is used for post-natal treatment, swelling, rheumatism. In Thailand they are applied for joint pain, intestinal disorders and numb feet (Sirirugsa, 1999). They were reported to have anti-fungal, anti-inflammatory, analgesic and antioxidant activity. This probably is due to the presence of certain secondary metabolites such as zerumbone, curcuminoid and (E)-1-(3,4-dimethoxyphenyl)but-1-ene (Kishore and Dwivedi, 1992, Ozaki et al., 1991, Habsah et al., 2000). There is a need to exploit its medicinal properties, therefore more planting material is needed. Slow propagation rate and the risk of disease transmittance through division by sectioning of the rhizomes have hampered propagation by conventional means. Thus in vitro technique is considered the best alternative that can supply a large number of planting materials for commercial planting and further study to discover their chemical properties.

MATERIALS AND METHODS

Explant sources and sterilization. The stock plants for this study were collected from Kampung Serambu, Bau District in the Kuching Division, Sarawak. Rhizome buds about 1 to 2 cm long were selected as the initial explants. The fresh buds collected were cleaned of soil dirt and left under running tap water for one to one and a half hour. Then the buds were immersed in 75% (w/v) ethanol for one minute. Without rinsing, they were agitate din 20, 30 or 40 % (w/v) Clorox (5.25 % w/v sodium hypochlorite) added with 0.1ml/L Tween 20 and four drops of 25% HCl for 20 minutes with constant agitation. After that they rinsed with sterile distilled water four times. Under aseptic conditions the bud scales were peeled off and then trimmed to about 0.5 cm long.

Culture medium. The medium used was the Gamborg B5 medium, gelled with 2.8g/L Gelrite and 30% sucrose as carbon source. The pH was adjusted to 5.7 -5.8 with 1N KOH or 0.1N HCl prior to autoclaving. Tetracycline at 15 mg/L and 2 ml/L Plant Preservative Mixture (PPM) were added to the medium to check the contamination. Trimmed buds were inoculated onto the medium and the cultures were

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