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Banana, gingers and papaya cell cultures for high throughput agriculture

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Abstract. There is considerable interest in developing plant cell cultures that could facilitate rapid propagation of elite materials and for the production of phytochemicals. In Malaysia, there is an urgent need for sufficient supply to meet the voluminous demand for seedlings which are important plantation and cash crops. Considering the cost of labour and infrastructure in conventional plantation, propagation in liquid culture is a practical way to produce clonal propagules at a low cost. Plant cell cultures could also provide an alternative source of phytochemicals. These cultures will ensure continuous high quality compounds and will also prevent the extinction of the valuable plants in their natural habitat. Alternatively, bioreactor could be used to maintain plant cell cultures on a large scale to enable the production of high quality cell lines of the major cash crops at a significant volume. One of the advantages of growing cell cultures in bioreactors is the opportunity to control critical parameters in order to reduce variations which could affect product quality and process reproducibility.

Besides being able to produce high-quality planting materials, plant cell cultures also allow genetic manipulation for trait improvement through either genetic engineering or mutagenesis (mutation breeding). Metabolomics is another possible endeavour in an attempt to enhance the production of certain fine chemicals in plants. This is to resolve the perpetual problem of non viable harvest of phytochemicals for commercialisation especially for the use of drug and product development .

In this paper, we will discuss the development of embryogenic cell suspensions of banana, papaya, and selected gingers (Boesenbergia rotunda, Zingiber zerumbet and Curcuma xanthorhiza) which have been field tested. The banana and papaya cell suspensions could produce up to 15, 000 plants and 7, 000 plants respectively for every 1 ml of settled cell volume within 5 -6 months. For the papaya and gingers, chromatographic spectrums of the phytochemical extracts from the cultivated type explants, tissue culture derived materials and cell suspension will be compared. The presence of the compounds in cell suspension will allow us to adopt genetic engineering technologies either to up-regulate or down-regulate certain enzymes for the enhancement of the targeted compounds in plants. In addition, physical parameters could be used to help increase the phytochemicals. For transgenic technologies, early flowering gene, Soc 1, has been successfully introduced into banana suspension but without the expected physiological changes when plants were transplanted.

Keywords: Banana; Papaya; Gingers; Cell suspension; Phytochemicals; Tissue culture; Micropropagation.

INTRODUCTION

The use of plant cell culture is an efficient method that could be adopted for micropropagation, secondary metabolite production and genetic manipulation. Plant cell cultures or cell suspension cultures are initiated either via embryogenic or non embryogenic callus. Embryogenic callus could form plantlets via somatic embryogenesis in contrast to non embryogenic which could be either be in differentiated or non differentiated callus forms. Somatic embryogenesis is a regeneration process that starts with non-zygotic cells transforming into pro-embryogenic mass, then bipolar somatic embryos and eventually forming plantlets. It is generally accepted that the demand of plant growth factors will vary accordingly, both in nature and concentration, as the asomatic cells differentiate into somatic embryos then into plantlets.

Very few reports have been published on the somatic embryogenesis of plants belonging to the ginger family (Malamug *et al*., 1991, Kackar *et al*., 1993, Salvi *et al*., 2001). Whereas the current protocols for somatic embryogenesis in banana are limited by low embryo development and plant recovery frequencies (Novak *et al*., 1989; Dhed'a *et al*., 1991; Côte *et al*., 1996; Becker *et al*., 2000; Ganapathi *et al*., 2001; Jalil *et al*., 2003).

As for suspension cultures of papaya, many protocols are developed for efficient micropropagation (Litz and Conover, 1983; Monmarson *et al*., 1995 and Jordan and Velozo, 1996). Besides complementing the conventional breeding (Minh and Thu, 2001), it is also used for genetic transformation purposes (Fitch and Manshardt, 1990). Current protocols are reported to be slow thus increasing the chances of somaclonal variation and were further hampered by the difficulty in rooting of the i*n vitro* plantlets.

In this study, embryogenic cell suspensions were developed for banana (*Musa acuminata* var mas), papaya (*Carica papaya* var eksotika 1) and selected gingers (*Boesenbergia rotunda*, *Zingiber zerumbet* and *Curcuma xanthorhiz*a). Protocols have been developed with the aim of increasing regeneration efficiency and simplicity in procedures. All of the protocols were developed for micropropagation and except for banana, all of the cell suspension were adopted for the production of sec-

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ondary metabolites such as carpaine from papaya, chalcones from *Boesenbergia rotunda*, zerumbone from *Zingiber zerumbet* and xanthorhizol from *Curcuma xanthorhiza*.

MATERIALS AND METHODS

The establishment of banana cell suspension is according to the method described by Wong *et al.,* (2006), using immature male inflorescence/bananas. Whereas the establishment of papaya cell suspension is according to Mohamad Fhaizal *et al* ., (2006) using zygotic embryos from immature seeds. As for gingers, active sprouting shoot buds were used as explants according to Tan *et al.,* (2005). Carpaine, zerumbone, xanthorhizol and chalcones were extracted using standard methods.

RESULTS AND DISCUSSION

In this study, a single medium formulation of Murashige and Skoog (MS) (1962) supplemented with 3 mg l-1 2, 4-D was found to be a suitable medium to promote the complete somatic embryogenesis process for the culture of *Boesenbergia rotunda*. The percentage of explants forming callus was 23.3 % ± 4.3 with a mean 6.6±0.1 plantlets per 1-cm diameter aggregate of callus. The regenerated plantlets have been successfully established in soil. As for *Zingiber zerumbet*, callus were initiated on MS basal medium supplemented with phytohormone and were transferred to M2D media (Cotes et al, 1996) for cell suspension maintenance after 4-8 weeks. Regeneration frequency from the cell suspension was approximately 210 plantlets/ml. Embryogenic callus (100%) was established from immature embryo of *Carica papaya* L. var. Eksotika I after 3-4 months of culture on Callus Induction (CI) medium. supplemented with 250 mg/L Carbenicillin plus 10 mg/L 2,4-D. Somatic embryos were maintained on a medium containing either reduced or without 2,4-D. The cultures could be maintained for a period of 5-6 months with no apparent loss of regenerative potential. The somatic embryos germinated on Germination (G) medium supplemented with 0.2 mg/L 6-Benzylaminopurine (BAP) and 2.0 mg/L 1-Napthaleneacetic acids (NAA) producing high regeneration frequency (88.41 %) with initial development of hypocotyls followed by rapid growth of plantlets. The *in vitro* shoots were readily rooted in G medium supplemented with 0.5 mg/L Indole-3-butyric acid (IBA) with 75 % successful rate. An improved method for high frequency recovery of banana plants was successfully formulated in this study by incorporating a liquid based, embryo-development media. The highest regeneration rate obtained using this liquid protocol was approximately 32 000 plants per ml. settled cell volume. This is one of the highest scores recorded among published data on plant recovery in *Musa* spp. The differentiation and regeneration period for most mature embryos was within 4 - 5 months.

Compounds extracted from rhizomes of the cultivated gingers, tissue culture derived rhizomes and cell suspension were found to be comparable but lower in amounts in the cell suspensions. Interestingly, in papaya cell suspension, carpaine were excreted out of the cells. As for plant transformation, from PCR, southern and western analysis, somatic embryos from bananas were successfully transformed with the early flowering *Soc* 1 genes. However, upon transplantation the banana plants did not flower after growing to maturity.

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