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Transformation of *Morinda citrifolia* Via Simple Mature Seed Imbibition Method

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Abstract: *Morinda citrifolia*, is a valuable medicinal plant with a wide range of therapeutic properties and extensive transformation study on this plant has yet been known. Present study was conducted to establish a simple and reliable transformation protocol for *M. citrifolia* utilising *Agrobacterium tumefaciens* via direct seed exposure. In this study, the seeds were processed by tips clipping and dried and subsequently incubated in inoculation medium. Four different parameters during the incubation such as incubation period, bacterial density, temperature and binary vectors harbouring β-glucuronidase (GUS) gene (pBI121 and pGSA1131), were tested to examine its effect on transformation efficiency. The leaves from the treated and germinated seedlings were analysed via Polymerase Chain Reaction (PCR), histochemical assay of the GUS gene and reverse transcription-PCR (RT-PCR). Results of the study showed that *Agrobacterium* strain LBA4404 with optical density of 1.0 and 2 h incubation period were optimum for *M. citrifolia* transformation. It was found that various co-cultivation temperatures tested and type of vector used did not affect the transformation efficiency. The highest transformation efficiency for *M. citrifolia* direct seed transformation harbouring pBI121 and pGSA1131 was determined to be 96.8% with 2 h co-cultivation treatment and 80.4% when using bacterial density of 1.0, respectively. The transformation method can be applied for future characterization study of *M. citrifolia*.

Key words: *Morinda citrifolia*, seed transformation, *Agrobacterium tumefaciens*, PCR, histochemical assay, RT-PCR

INTRODUCTION

Plant genetic transformation is an approach used to introduce foreign gene in plants (Chawla, 2002). Techniques in plant transformation have been widely established. Among others, the most frequently utilised methods are the Ti-system of *Agrobacterium tumefaciens* (Mohamed *et al*., 2006; Gonzalez *et al*., 2008; Ogaki *et al*., 2008; Yasmeen *et al*., 2009; Xu *et al*., 2009a, b), microinjection of DNA (Evans, 2006), polyethylene glycol mediated DNA uptake (Mathur and Koncz, 1998), the use of plant virus vectors (Scholthof *et al*., 1996; Nagl *et al*., 2005) and particle bombardment of naked DNA or plasmids into plant organs and protoplasts (Ueki *et al*., 2008). Traditionally, plant transformation have successfully utilised the tissue culture method but the drawback in using tissue culture-based transformation is the possibility of increased contamination in nutrient-rich media and somaclonal variation in transgenic plants due to the long exposure with growth hormones. Feldman and Marks (1987) have successfully used *Arabidopsis thaliana* seeds to generate transgenic plants. Subsequently, Bechtold *et al*. (1993) developed the widely used in planta transformation method to deliver foreign gene into plants. The direct seed transformation utilising *A. tumefaciens* is a method that bypasses the tissue culture method which is tedious, time-consuming and prone to contaminations and variations (Slater *et al*., 2005; Yasmeen *et al*., 2009). *A. tumefaciens* is a natural target of dicotyledonous plants. Since, its introduction, direct seed transformation has been applied to various plants, such as *A. thaliana* (Feldman, 1995), *Zea mays* (Wang *et al*., 2007) and *Brassica napus* (Song *et al*., 2009). Transformation of *A. thaliana* was conducted using pre-imbibed seeds in a co-cultivation medium containing fresh log phase *A. tumefaciens* culture (Feldman, 1995) whereas in maize, the transformation was conducted using germinating seedlings with cut wounds at the apical meristem (Wang *et al*., 2007).

Morinda citrifolia originated from the division Magnoliophyta, class Magnoliopsida, order Rubiales and family Rubiaceae. It is a small evergreen tree, native to the Southeast Asia and Australian region (Nelson, 2006) with the variety bracteata indigenous to Malaysia and Indonesia. The plant is usually 3-10 m tall and has the advantages in that it thrives on non-fertile, acidic or alkaline soils and can withstand adverse environmental conditions such as wind, fire, drought and flooding (Nelson, 2006). There are three main varieties of

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