Introduction

Papaya (*Carica papaya* L.) is one of the most widely grown crops in the tropical and sub-tropical region (Khuzhaev and Aripova, 2000 and Azarkan *et al*., 2003) including Malaysia, where it is a smallholders' crop (Vilasini, 2000). This is because of its high demand as a multi-purpose fruit, not only as dessert fruits but also as a source for chemical compounds for medicinal use like papain, chymopapain and carpaine (Dawson, 1998 and Litz, 1983).

Many hybrids of papaya have been developed world wide for commercialization, for instance Honey Dew, Kapoho, Setiawan, Solo, Subang, Sunrise, Sunset, Sunrise Solo, Tainung, Waimanalo, and Eksotika. There are several cultivars of papaya, both locally developed and introduced in Malaysia.

Eksotika is the main variety grown for export in Malaysia. This variety was developed and introduced by Malaysian Agricultural Research and Development Institute (MARDI) in 1987 to promote the development of papaya industry. The improved characteristics of this variety include fruit texture, taste, size, and also tolerant to Papaya Ring Spot Virus (PRSV) with good post-harvest quality to enable long transportation (Chan and Teo, 1994).

Traditionally, papaya planting materials are obtained through seeds and conventional breeding via seed is carried out for cultivar improvement. However, this might lead to low productivity due to miss-selection of planting material, poor cultivation techniques and management practices. Alternatively, various tissue culture systems have been developed for micropropagation in order to overcome the limitation in conventional breeding.

Papaya tissue culture researchers have reported plant regeneration from different explants of popular varieties, both through organogenesis and embryogenesis (Litz and Conover, 1983; Chen *et al.* 1987; Fitch and Manshardt, 1990 and Hossain *et al.* 1993).

It has been reported that papaya explants have been regenerated via somatic embryogenesis using mature embryo (Litz and Conover, 1983), anther (Tsay and Su, 1985, and Rimberia *et al*., 2005), hypocotyls (Jordan, 1986; Fitch, 1993, and De Almeida *et al*., 2000), root (Chen *et al*., 1987, and Yu *et al*., 2003), seed (Yamamoto and Tabata, 1989), immature embryos (Fitch and Manshardt, 1990; Chen and Chen, 1992; Castillo *et al*., 1998; Sutanto *et al*., 1999; Vilasini *et al*., 2000; Yu *et al*., 2001; Fernando *et al*., 2001; Renukdas *et al*., 2003 and Bhattacharya *et al*., 2003), *in vitro* lamina and stem (Mondal *et al*., 1994), integuments of immature seeds (Monmarson *et al*., 1995), and axillary buds (Jordan and Velozo, 1996).

Somatic embryogenesis via suspension cultures of papaya was also carried out by many researchers for an efficient micropropagation protocol (Litz and Conover, 1981; Jordan, 1986; Chen and Chen, 1992; Jordan and Velozo, 1996; Castillo *et al*., 1998 and Sutanto *et al*., 1999).

To date, immature embryos are the most common tissue used for the propagation of cell suspension of many papaya species. However, current protocols developed using this explant were reported to be slow and is further hampered by the difficulty in rooting of the *in vitro* plantlets. The success of papaya tissue culture is greatly dependent on the culture medium used and particularly the exogenous application of

plant growth regulators such as 2,4-D, BAP, IAA, IBA, GA_3 and NAA. Nutritional requirements for optimal growth may vary with the species.

In this project, experiments have been carried out to improve the existing published protocols for embryogenic callus induction, development of cell suspension for embryo multiplication and finally the regeneration of complete papaya plants.

In herbal medical practice, 5000 alkaloids of all structural types are known and being explored. Alkaloids as secondary metabolites could be classified as true alkaloids, proto alkaloids, and pseudo alkaloids and were explored since 1819 (Rajnikant *et al*., 2005).

Among the secondary metabolites classified as alkaloids in papaya plants, carpaine, is the lead compound found in root, seed, bark, fruit peel, and leaves ranging from 1000 to 1500 ppm as reported by Burdick, 1971.

Studies on various aspect of carpaine have been carried out by many researchers (Rajnikant *et al*., 2005; Khuzhaev and Aripova, 2000; Jacques *et al*., 1997; Jacques *et al*., 1994; Michel and Eric, 1985; Soedinenii, 1983; Eric, 1981; Tang, 1978; Eric, 1976; Brown, 1975; Corey, 1975; Brown, 1973; Brown, 1972; Burdick, 1971; Ogan, 1970; Coke and Rice, 1968; Rice, 1967; Bevan and Ogan, 1964; Govindachari and Narasimhan, 1953; Rapoport and Baldridge, 1952 and Barger *et al*., 1937).

Analysis of carpaine from *Carica papaya* L. plants from various plant parts was carried out from separate studies; seed (Farias *et al*., 2007; Cheng and Tsai, 2004; and Wilson *et al*., 2002); fruit (Nitsawang *et al*., 2006; Azarkan *et al*., 2003; Knez *et al*., 2003; and); root (Tang and Takenaka, 1983) and leaves (Khuzhaev and Aripova, 2000;

Tang, 1978; Burdick, 1971; Ogan, 1970; Coke and Rice, 1965; Bevan and Ogan, 1964 and Govindachari and Narasimhan, 1953). However, in this study, carpaine was quantitatively determined from all plant parts of *in vivo* and *in vitro* origin and cell cultures.

The chemical configuration of carpaine is a pyrolidine structure with a lactone moiety attached to the α-position (Govindachari, 2002) and consists of two identical substituted piperidine rings bridged by two ester groups (Burdick, 1971). The model suggested by Govindachari and Narasimhan, 1953 indicated that the dimeric carpaine molecule is flexible and the two piperidine rings could assume the chair forms without restraint (Rajnikant *et al*., 2005) as shown in ***Figure 1.1***.

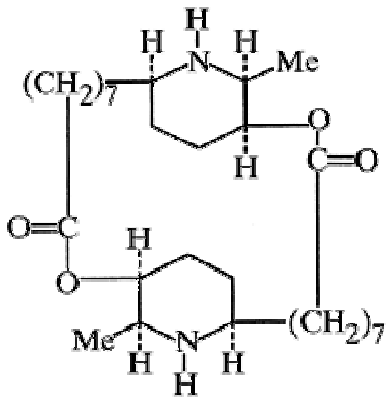

Figure 1.1: Chemical structure of carpaine

Preceding studies have reported that the extraction of carpaine from papaya leaves inadvertently include some impurities such as ψcarpane, ψcarpamic acid, ψcarpaine (Govindachari, 2002), pseudocarpaine (Khuzhaev and Aripova, 2000; Ogan, 1970; and Govindachari and Narasimhan, 1953), novel carpaine-derived macrocyclic ethers (Jacques *et al*., 1997), carpaine monoamides (Jacques *et al*., 1994) and dehydrocarpaine I and II (Tang, 1978). Hence, only a small amount of carpaine thrives to be extracted each time. In this study, an improved conventional liquid-liquid

acid base extraction method was developed to extract the right carpaine compound (Khuzhaev and Aripova, 2000; Tang, 1978; Ogan, 1970; Burdick, 1971; Coke and Rice, 1968 and Govindachari and Narasimhan, 1953). However, following the supercritical fluid extraction (SFE) method was also developed for carpaine extraction by Knez *et al*., 2003.

It is critical that the right carpaine compound is obtained, in order to qualitative analysed using an internal standard method of NMR (nuclear magnetic resonance) and gas chromatography-mass spectrometry (GC-MS).