

Abstract

Carica papaya L. is a member of *Caricaceae* family with four native genera. This fruit is economically important as in multi-purpose fruit produced for dessert and vegetable and is highly in demand for pharmaconomical trade particularly the plant compound as source of medicine. This species is a popular fruit crop in the tropical and sub-tropical regions of Malaysia, Thailand, Indonesia and the Philippines. It is also one of the most widely grown fruits in Malaysia and is mainly cultivated in Johor, Perak and Selangor. The Eksotika papaya variety was introduced to the farmers in the late eighties and this resulted in an increase in the production areas.

Therefore, the innovation of getting sufficient and superior material has to be made in order to fulfill the high demand of superior planting material and also as a source of material for the purpose of further research and development of *Carica papaya* L. cell cultures and for its bioactive compound. Papaya tissue culture researchers have reported plant regeneration from somatic embryos and it has been confirmed that papaya responds well to somatic embryogenesis from immature zygotic embryo. In order to be successful in extracting compounds from *in vivo* and *in vitro* samples, an efficient regeneration system has been in place for the cultivar Eksotika. In this connection, prior to isolation of compound, an efficient plant regeneration system of *Carica papaya* L. var. Eksotika from suspension cultures need to be developed. This is to facilitate sufficient material resources for extraction. The idea of using embryogenic cell suspension cultures is to produce complex chemicals as pharmaceuticals. The current work obtain the establishment of cell suspension cultures for the production of valuable chemicals and the work now is to find the right culture conditions that will allow establishing plant cell cultures to produce valuable chemicals.

Explants of immature zygotic embryo excised from *Carica papaya* L. variety Eksotika placed on callus induction medium supplemented with 250 mg/L carbenicillin induced callus at 93.3 ±11.8% after 14 days of culture. Furthermore, by using double staining method, nuclei of embryogenic cells were stained and identified as intense bright red colour. Embryogenic cells were selected and used for cell suspension cultures. Further verification was showed through the histological structure of the embryogenic cells. Groups of small meristematic cells in the whitish parenchymatic microcalli were observed followed by embryo development. In addition, the best mediaum for the induction of somatic embryos was MS basal medium supplemented with 10 mg/L 2,4-D and 250 mg/L carbenicillin where 80.4 % of the callus gave rise to somatic embryos. Subsequently, the multiplication of cells in suspension cultures was initiated in liquid multiplication medium containing 250 mg mg/L carbenicillin. A growth pattern was observed where the lag phase was observed from day 10 to day 19, followed by an exponential growth phase from 20 day to 30 day. Cell viability assessment using fluorescence diacetate solution showed that only cells with dense cytoplasm fluoresced under ultraviolet light excitation. These cells were small, rounded and with distinct nucleus. The germination medium was supplemented with 0.2 mg/L BAP and NAA was the optimal culture media where 100 % somatic embryo germination was observed. Synchronous germination of somatic embryos was obtained from suspension cultures where hypocotyls initially became swollen followed by rapid shoot multiplication. Shoots were rooted on regeneration medium supplemented with 1 mg/L GA₃, 0.5 mg/L IBA and 0.37 mg/L riboflavin. Finally, during acclimatization, when the first set of matured tri-lobed leaves appeared, plantlets were transferred to the nursery for one to two months before transplanting to the field.

Due to the ease of the isolation procedures, many of the earliest pure compounds isolated with biological activity were alkaloids. The nitrogen molecules in plant cells generally make

the compound basic which make alkaloids exist in a salt form in plants. Thus, alkaloids are often extracted with water and/or acid solution system and then recovered as crystalline material by treatment using a base.

The alkaloid carpaine was extracted from various part of *Carica papaya* L. var. Eksotika from field grown samples namely leaves, petiole and fruit peel, and from *in vitro* samples namely leaves, petiole, suspension cells and suspension liquid with only one artifact of impurity detected i.e. dehydrocarpaine II. Supercritical fluid extraction was analysed to obtain pure and high yield of carpaine compound as compared to conventional acid base extraction method. The ratio of ethanol/water/acetic acid used at 94.5:5:0.5 (v/v/v) was confirmed to be a better solvent system for carpaine extraction since only one pseudocarpaine i.e. dehydrocarpaine II was extracted together with carpaine. In this study, the application of single fluid of carbon dioxide in supercritical fluid extraction procedure generated pure and higher yield of carpaine compound. Additional centrifugation step should have contributed to a higher purity of the extracted carpaine.