Chapter IV

Discussion

Selection internodes of *Nyctanthes arbor-tristis* L. used as a source of explants in this work. After surface sterilization, the explants were necessary to standardize the sterilization procedure for each starting material before the beginning of experiment. Surface sterilization condition were always necessary and might be carried out with any several different reagents (Yeoman et, al, 1977). The internode section were the best part for rapid propagation of plant in a short period of time.

Nutritional requirements for growth and development of plant tissue *in vitro* culture might be varied with certain species. Even the tissues from different parts of the same plant may differ in nutritional requirement for satisfactory growth (Murshige and Skoog, 1962). Composition of basal culture medium was an important factor for the successful establishment of tissue culture. Therefore each tissue culture required different formulation (Dixon, 1987). This might be due to the difference of macronutrient and micronutrient composition in each medium (George and Sherrington, 1984). As evidence in Table 3.1, it was noticed that the various media were tested for cultivating of *Nyctanthes arbor-tristis* L. For internode segments, MS medium supplemented with 0.4 mg/l BA was the most effective in the shoot induction and growth. MS medium supported a good growth and gave satisfactory shoot induction efficiency. Generally, MS macronutrients were developed and proved to be satisfactory for tissue culture of many plant species for

micropropagation (Murashige and Skoog 1962). Most of other media were developed based on the composition of original MS macronutrients, such as Hasegawa (1979), Jarret et al. (1980). The other media, which were tested for culturing of *Nyctanthes arbor-tristis* L. yielded lower response for shoot induction and growth development in comparison to the MS medium. The reason was a large range diversity in the concentration of the nutrient components. Multiple shoots were induced with BA 0.4 mg/l and NAA 0.1 mg/l. However the multiple shoots were reduced when NAA was increased 0.2, 0.3, and 0.4 mg/l. The obtained data was similar to those of Lakshmi (1986) and found that lateral bud of *Eucalyptus grandis* in MS medium with BAP 1 mg/l and NAA 1 mg/l were induced for multiple shoots. The results showed that NAA was added to the medium, then shoot formation was completely suppressed. While BA in combination with some auxins presented in the induced medium for multiple shoots of *N. arbor-tristis* L. It was suggested that multiple shoots or shoot formation was highly dependent upon a function of different kind of cytokinin. Similar results were observed by Sangwan and Harada (1976), Vieitez (1980). The similar effect of interactions between auxin and cytokinin for somatic embryogenesis had been reported with other systems such as Begonia (Wesander, 1977) and *Arabidopsis thaliana* (Patton and Meinke, 1988). The present work also showed that root formation observed only on shoots cultured with low levels of 2,4-D 0.1 mg/l. However, the multiple shoots were developed on MS medium and various BA, and NAA supplement with activated charcoal produced shoot formation. But this medium failed to developed into normal plantlets. Even through, root

induction was occurred in only MS medium with 2,4-D and not found in 2,4-D with activated charcoal.

Crude extracts from leaves and stems of mother plants from *Nyctanthes arbor-tristis* L. were shown the antimicrobial activity on agar medium. They were antibacterial activity in gram negative (*E. coli*) and gram positive (*Bacillus substilis*) to 0.15 mg/ml and 0.075 mg/ml. Crude extracts were resistance to *C. cerevisiae and aspergillus sp.* in only 0.15 mg/ml. Crude extracts from leaves and stems of tissue culture were shown antimicrobial activity for *E. coli*, *B. substilis* and *S. cerevisiae* to 0.15 mg/ml. However, they were not resistance to *Aspergillus sp.* Arya and Daniel (1997) studied on the fungal infected leaves of *Nyctanthes arbor-tristis* L. revealed that the flavonoids and phenolic acids got converted or degraded as a result of the pathogens. In Crataeva the flavonoids, such as quercetin and 3-θ quercetin showed degradation to trace levels. In *N. arbor-tristis* L. got methylated to less toxic 4'-θ kaempferol. Syringic acid in lignin biosynthesis was also degraded to a great extent as a result of infection. Similar effect had been reported with Talakal et al (1995) on antimicrobial activity. They studied aqueous extracts of 9 indigenous plant materials were screened *in vitro* for their activity against *Trypanosoma evansi* at concentration of 5, 50, 500 and 1,000 µg/ml. The extract of *Nyctanthes arbor-tristis* L. at a concentration of 1,000 µg/ml was highly effective. Nadkarni (1976) revised that indigenous plants were exploited successfully against a number of protozoa infection, viz. *Plasmodium falciparum*, *Leishmania donovani*, *Entamoeba histolytica.* Indigenous plants, viz. *Achyranthus aspera* leaves, *Azadirachta indica* leaves, *Cassia occidentalis* leaves, *Nyctanthes arbor-tristis* L. leaves, claimed to be

useful as antipyrectic, antiprotozoal, anthelmintic etc. The present work also showed that the crude extract from leaves and stems (mother plants) were shown two spots with Rf value of 0.22 and 0.27 respectively in solvent systems (II). The response to the test for β-sitosterol (changed colour green-blue) and trierpenoid (changed colour red-pink) was positive respectively from Libermann-Burchards test method. After that crude extract from leaves and stems (tissue culture) were shown a spots with Rf value of 0.36 in solvent systems (II). The response to the test for β-sitosterol (changed colour green-blue) was positive respectively. However, all samples were not responded to test for iridoids. This might be due to the double form of mother plants were modified for alkaloid substance. On the other hand, the single form of tissue culture were not developed completely for alkaloid substance. H. Stuppner et, al (1992) had been reported that the isolation of three iridoid glycosides from the leaves of *Nyctanthes arbor-tristis* L., 6,7-di-O-benzoylnyctanthoside, 6-O-trans-cinnamoyl-6B-hydroxyloganin and 7-O-trans-cinnamoyl-6B-hydroxyloganin. These structures were established by UV, mass spectroscopy and NMR spectroscopy. Finally, the growth inhibition activity from mother plants and tissue culture (leaves and stems) on *E. coli, B. substilis*, were inhibited growth and *S. cerevisiae* and *Aspergillus sp.* were partial inhibited growth. The growth inhibition in bacteria was better than in yeast and mold. Dilution extract was affected the growth inhibition.

Carotenoids, which were considered to be saved and stabled, were currently used primarily as colorants in food products such as margarine. However, there was increasing interested in their possible role in maintaining health particularly in regard to lowering the risk of cancer. Many multivitamin

supplemented now containing β-carotene. The present work also showed that the extraction of carotenoid from flower stalk of *Nyctanthes arbor-tristis* L. were found 844 mg/kg by TLC with Rf = 0.93. Commercial synthetic carotenoids are mainly used as pigments for food (egg yolk, chickens, or farm-raised salmon) and for coloration of food products (margarine, cheese). The β-carotene was widely used for coloring soft-drink, friuit juices, breads, cream-cakes, fishes products, ice-cream, etc. as well as a nutritional supplement (Xihai, 1990).