CHAPTER I

INTRODUCTION

A characteristic feature of higher plants is their ability to biosynthesize a wide variety of natural products, that so-called " secondary metabolites ". For a long time these compounds were regarded as waste products which had interesting structures and, in many cases, exploitable biological properties. However, a rapid increase of experimental evidence indicates that most secondary metabolites are important for the overall fitness of the plant that produces them. Their major functions include defence against herbivores (insects, mollusce, vertebrates), microorganisms (viruses, bacteria, fungi). In addition, some secondary metabolites display metabolic or further ecological functions, for example, UV-protection, attraction of pollinating or seed-dispersing animals (Levin, 1976 ; Swain,1977 ; Rosenthal and Janzen,1979 ; Harborne,1982 ;Wink,1988). Since plant secondary metabolites have evolved as factors in the interaction with other organisms, many of them have interesting biological or therapeutical activities. As a consequence, a number of these compounds are economically important, serving as pharmaceuticals, aromatics, fragrances, stimulants, colors, and pesticides.

Despite advances in the field of organic chemistry, plant are still an important commercial source of chemical and medicinal compounds. In most cases, however, these plants have not been subjected to intensive genetic programs for optimum production of the compounds. In addition, there have been technical and economic problems in the cultivation of these plants (Shuler,1981). Since plant cell isolated from field-grown plants and cultivated *in vitro* have the potential to produce and accumulate chemical identical with those produced by the parent plant, plant cell cultures have been considered as an alternative means of producing commercially important secondary metabolites.

The potential advantages of the *in vitro* cultures over traditional field methods of cultivation are clear, in particular, independence from geographical, climatic and political problems (Fowler,1983). In addition, with plant cell cultures, it may be possible to optimize growing conditions, minimal space requirement, achieve more consistent quality and recover the products more easily (Shargool, 1982).

Undoubtedly, cell culture could provide the continuous, rather homogenous supply of plant material of a defined physiological state required by commecial industry (Berlin, 1986). The main question, however, is how the specific physiological state necessary for a high rate of formation and accumulation of a desired product can be established in cultures. Over the last decades, relatively few cell cultures have been established which can produce substantial amounts of plant secondary metabolites (Staba, 1980 ; Berlin, 1986). There are numerous reasones why progress has been slow in the industrial application of cell cultures for the production of secondary metabolites. The cultures exhibit relatively slow rate of growth, and the biosynthesis of the desired compound is often at much lower level than present in the intact plants. On the other hand, accumulation of secondary metabolites may also require specialized storage cells which may not be identical with the synthesizing cells. Since tissue culture techniques have been optimized for maintenance for a high rate of growth and division, which tends to limit cell differentiation and development of organized tissue systems, it is not surprising that many secondary metabolites are produced little or not at all in cultures.

Another main reason of the slow progress of the biotechnological production of secondary metabolites by plant cell cultures is that the knowledge of biosynthetic enzyme pathways and regulatory aspects of secondary metabolism in higher plants is rather limited. The biochemical reasons are almost completely unknown why one pathway is expressed in one organ for a certain period of time while another is inactive at that time.

To manipulate plant secondary metabolism according to our needs, we have to understand the basic principles of biochemistry of product formation. This could only be

accomplished by using techniques of enzymology which involve purification and characterization of the biosynthetic enzymes.

Among various secondary pathways in plants, the biosynthesis of naphthoquinones is of particular interest. There are at least four different groups of naphthoquinones classified based on their biosynthetic pathways. In this study, emphasis was put on the naphthoquinones found naturally in *Impatiens balsamina* L. plant. These are 2-hydroxy-1,4-naphthoquinone (lawsone) and its methyl ether, 2-methoxy-1,4-naphthoquinone (Little *et al.*, 1948 ; Bohm and Towers, 1962). Both lawsone and its methyl ether have been reported to exhibit strong antifungal activities (Steffen and Peschel, 1975 ; Tripathi *et al.*, 1978 ; Thatree Phadungcharoen, 1988). The fungitoxic property of these naphthoquinones has been explained as a short circuiting of the cell electron transport normally executed by quinones (Holmes *et al.*, 1964 ; Kelkar, 1986 ; Monthon Sanguansermsri,1991)

Biosynthetically, it has been purposed based on feeding experiments that lawsone is formed in plant *via* 2-succinylbenzoic acid (Dansette and Azerad, 1970 ; Grotzinger and Campbell, 1974), a key intermediate arise from glutamic acid (Chen and Bohm, 1966 ; Herbert, 1981) and chorismic acid (Herbert,1981). However, none of the enzymes involved in the formation of this intermediate have been isolated. Also, the biosynthetic sequence from 2-succinylbenzoic acid to lawsone is still not clear although it has been purposed that no symmetrical intermediate (e.g. 1,4-naphthoquinone) is involved in lawsone biosynthesis (Grotzinger and Champbell, 1971).

As part of our interest in elucidation of the biosynthetic pathway of lawsone and its methyl ether, we first aim to search for a suitable source of starting material used for the study. Since plant cell cultures rather than differentiated plants have been proved to be preferred material for studying secondary product formation and elucidating the enzyme pathway (Zenk *et al.*, 1985), we decided to establish various *in vitro* cultures of *I. balsamina* and evaluate for their potential in synthesizing lawsone and its methyl ether. In

this report, the characteristics of each type of *I. balsamina* cultures and some of its growth and production patterns are presented.