STUDIES ON THE BIOCHEMISTRY OF GALL FORMATION IN CINNAMOMUM TAMALA FR. NEES WITH SPECIAL EMPHASIS ON AUXIN METABOLISM

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THESIS SUBMITTED IN FULFILMENT OF THE DEGREE OF

DOCTOR OF PHILOSOPHY IN BOTANY

To

THE NORTH-EASTERN HILL UNIVERSITY

SHILLONG, INDIA

SEPTEMBER, 1986

DEDICATED

TO

MY PARENTS

ACKNOWLEDGEMENT

I wish to express my deep sense of gratitude to my teacher Dr. P. Tandon, Reader, Department of Botany, North-Eastern Hill University, Shillong for his able guidance and continued keen interest during the course of this investigation.

I am grateful to Prof. R.S. Tripathi, the present Head of the Department of Botany, and Prof. P.S. Ramakrishnan and Prof. R.R. Mishra, former Heads of the Department, for providing necessary laboratory and research facilities.

My sincere thanks are also due to Dr. T.S. Rathore, and my fellow research workers Messrs A.N. Verma, S.K. Sharma, A.L.S. Rajee, B.B.Das, J.C. Dang and Miss J. Sharma for their generous help in many ways in preparation of this thesis. To Dr.(Mrs.) V. Tandon, I owe a special word of thanks for her help.

I wish to acknowledge the assistance from Mr. B. Das for photography, Mr. S. Choudhary for drawings, and Mr. G. Pathaw and Mr. N.K.Paul Choudhury for typing the manuscript.

I also thank the teaching staff and members of the Department of Botany for their help and cooperation.

Words are inadequate to express my deep sense of gratitude to my parents who have always been a source of constant inspiration in my research endeavour.

Financial assistance in the form of Junior and Senior Research Fellowships from Council of Scientific and Industrial Research, New Delhi, India, for carrying out this work is thankfully acknowledged.

SHILLONG
September 26, 1986

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CHAPTER I

GENERAL INTRODUCTION : REVIEW OF LITERATURE

Plant galls have aroused the curiosity of many investigators since the early christian era. The galls were used primarily for medicinal and tanning purposes (Wittlake, 1981). A large number of plants are known to show gall formations. The various agents or conditions known to incite tumorous growths in plants are: physical and chemical agents, genetic constitution, biological agents (phytocecidia, zoocecidia and plasmatocecidia) and circumstances of life (Arya **et al.**, 1975; Paclt, 1981; Bayer, 1982).

Plant tumor is an abnormal growth. Most of the abnormal growths are characterized by extensive alterations and over growths due to the plant organs losing control over the growth potential of the affected area. These abnormal growths strikingly illustrate the ability of an infectious agent to interrupt normal plant morphogenetic processes and re-establish growth in a new and stable state that is potentially beneficial to the pathogen.

Plant tumors are of two main types: i) benign tumors (self-limiting) which grow slowly and remain localized in the host and ii) malignant tumors (non-self-limiting), that are composed of cells showing uncontrolled growth and invade neighbouring tissue and spread throughout the body of the

organism by metastasis (Braun and Stonier, 1958).

Man has always had his troubles with insects or mites. Approximately 15,000 gall forming insects have been recorded from around the world (Rohfritsch and Shorthouse, 1982). It has been estimated that approximately one-third of the world's crop is damaged or destroyed by insects or mite pests, either directly by their feeding (phytophagous) or by transmitting other disease causing agents when they feed during growth, harvest and storage of crop plants (Jacobson, 1982; Coulson and Witter, 1984). Losses are even higher in many under developed countries. The monetary loss due to feeding by larvae and adult of insect pests amounts to billions of dollars each year (Jacobson, 1982).

Insect/mite-incited galls, commonly known as zoocecidia, are reported to be self-limiting, and continued presence of insects or mites is considered essential for gall tissue growth (Braun, 1969). These galls may be simple involving a single organ, or compound, where two or more plant organs are concerned in the production of galls. Some of the best known cecidozoans are the ones belonging to Acarina like **Eriophyes**, and insects belonging to Thysanoptera, Psyllidae, Homoptera, Coleoptera, Hymenoptera, Cecidomyiidae and Diptera etc. (Mani, 1973).

Küster (1911) distinguished two kinds of galls on

the basis of their structure i) the kataplasmas, caused by many thrips and homopterans, and characterized by less differentiated tissue and lack of constant external shape and ii) the prosoplasmas, caused by diptera and cynipidae and characterized by having well-defined zones and a definite shape and size.

Gall forming insects are generally considered to be host specific. Some gall formers attack only one host species, while the others are restricted to a few closely related species within one genus (Rohfritsch and Shorthouse, 1982). However, Westphal (1980) has observed non-specific mites (**Eriophyes cladophthyrus**) which provoke gall formation on species as different as **Solanum, Nicandra,** and **Petunia.** Jeppson **et al.** (1975) reported a relatively wide range of host plants for two eriophyids. Ananthakrishnan and Raman (1977) summarized the knowledge pertaining to host specificity and selection by gall inducing thrips, coccids, midges and aphids. In general, the chemical or physical basis of such host specificities are unknown. In some cases, however, specificity or selectivity is related to the production and release by the pathogen of toxic substances with remarkable characteristics. These are so called 'host specific toxins' which damage or destroy the host plant tissues that are susceptible to the toxin-producing microorganisms, but have little or no effect on other plants, microorganisms or animals

(Browder and Eversmeyer, 1986). These toxins are considered to be essential for pathogenicity.

A vast descriptive literature explains how these specialized insects called 'cecidozoans' structurally alter their host organs, but the process still remains poorly understood. A hypothesis given by Rahn (1936) suggested that mitogenetic rays given by the larvae caused abnormal proliferation. Black (1945) concluded that insect galls may be formed by certain viruses associated with the insect larvae. This view gained strength with the discovery of plant tumors caused by an insect-transmitted virus, **Aureogenus magnivena.** Parr (1940) stated that the stimulus for gall production by insect is chemical. Attempts have been made to extract the gall forming substance or substances from the pathogen. The salivary secretion was injected into the host tissue to reproduce galls artificially. Several substances, including growth hormones, amino acids, amides, steroids and numerous digestive enzymes, have been detected in the saliva of cecidozoans (Küster, 1911; Anders, 1961; Hori, 1974; Byers **et al.**, 1976; Hori and Miles, 1977; Rohfritsch and Shorthouse, 1982; Dixon, 1983). Anders (1961) has presented evidence about the presence of proteolytic enzymes like protease and peptidase in aphid saliva and has emphasized their role in cecidogenetic changes in host tissue.

Lewis and Walton (1958) showed that the formation of cane-gall of witch hazel, **Hamamelis virginiana** L. resulted from the injection of 'cecidogen' into the plant by the aphid, **Hormaphis hamamelidis**. Later in 1964, these authors stated that leaf galls on **Celtis occidentalis** were caused by the injection of cecidogen into the leaf by a psyllid (**Pachypsylla** sp.). Galls caused by mites, midges, wasps, aphids and other arthropods demonstrated the presence of cecidogen in both insect and gall tissues. Cecidogen occurs as amorphous masses, crystals or granules and multiplies in the insect but not in the plants and moves from cell to cell in both animal and plant tissues. The cecidogen was considered a virus-like material because of its similarity with viruses found in kataplasmic galls (Walton, 1980). Boysen-Jensen (1948) concluded that gall formation was caused by growth regulatory substances secreted by larvae at different loci. McCalla **et al.** (1962) showed that unidentified adenine derivatives, glutamic acid and possibly uridine were also present in the salivary glands of **Pontania pacifica** which produced galls in willow (**Salix alba** L.). They further stated that these substances were of physiological importance in gall development. However, biochemical and physiological aspects of insect/mite-incited gall formation in plants have received little attention as compared to crown gall.

Crown gall, a non-self-limiting tumor on many plants, is induced by a virulent strain of a gram-negative, soil bacterium, **Agrobacterium tumefaciens** (Smith and Townsend, 1907) Conn. The majority of host species belong to the dicotyledons, but infection in some monocotyledons has also been reported. The crown gall disease has been intensively studied (Braun and Stonier, 1958; Butcher, 1973; Meins, 1974; Lippincott and Lippincott, 1975, 1976; Kado, 1976, 1984; Braun, 1982; Kahl and Schell, 1982; Roberts, 1982; Binns, 1984; Nester **et al.**, 1984). It is well known that during the induction of crown gall, the T-DNA from the Ti-plamids (Thomashow **et al.**, 1980) is transferred to the nucleus of the normal plant cell (Schell **et al.**, 1979) where it is covalently joined to the host plant DNA (Yadav **et al.**, 1980), maintained (Drummond **et al.**, 1977; Chilton **et al.**, 1980) and transcribed (Drummond **et al.**, 1977) in the transformed plant cells. In addition to its oncogenetic properties, the T-DNA is essential for the maintenance of the tumorous state and for the biosynthesis of a group of unusual plant metabolites called opines (Holster **et al.**, 1980; Tempe and Goldmann, 1982) which serve as a source of energy and nutrients for the bacteria (Tempe and Petit, 1982).

It has been found that during transformation of a normal plant cell to tumor cell, the biosynthetic systems

concerned with cell division and growth are persistently activated. This results in the production of significant amounts of cell-division promoting substances as well as other essential metabolites which play a central role in development of a capacity for autonomous growth of the tumor cells (Braun and Naf, 1954; Stonier, 1972; Miller, 1974; Fracassini **et al.**, 1980; Bouckaert-Urban and Vendrig, 1981; Amasino and Miller, 1982; Braun, 1982; Kado, 1984).

The various aspects of physiological differences in tumor cells with respect to normal cells have been well summarized (Braun and Stonier, 1958; Braun, 1962, 1982; Klein, 1965; Kado, 1976). The literature on crown gall suggests two fundamental alterations which may underlie the tumorous state: changes in energy metabolism resulting in a more highly reduced and more anaerobic state and changes in cell permeability (Lippincott and Lippincott, 1975). Kehr (1965) reported that genetic tumors were initiated by physiological processes which were without doubt caused by gene action affecting the internal metabolic systems of the organism. Braun (1957) demonstrated the interaction and interdependence of several distinct metabolic systems which affect abnormal growth.

Plant galls have been reported to contain higher levels of growth hormones (Byers **et al.**, 1976; Purohit

et al., 1980; Tandon and Arya, 1980a; Weiler and Spanier, 1981; Braun, 1982; Malan et al., 1982; Pengelly and Meins, 1982; Dixon, 1983; Kado, 1984; Madden and Stone, 1984; Nester et al., 1984) which presumably contribute to the abnormal growth. It is now generally believed that crown gall tumor transformation is a two-step process. During tumor inception, foreign genes are introduced into the plant cell, and these genes result in the commitment of cells to a pattern of autonomous growth. The second step, auxin requiring, is the expression of the committed state, which may have an epigentic basis (Braun, 1978). Physiological changes occurring in diseased tissues can be conveniently ascribed to alterations in auxin levels. Even when such alterations can be demonstrated, it is never entirely clear as to which is the cause or which is the effect of the growth changes thus produced? This is because of the factors other than, or in addition to, auxin that may mediate many of the exaggerated growth responses characteristic of plant diseases (Bouckaert-Urban and Vendrig, 1981).

The increased levels of indole-3-acetic acid (IAA) in diseased tissues pose a problem regarding its origin. Klein and Link (1955) have suggested that the bacteria, which are known to synthesize auxins (Bertholet and Amoureux, 1938), are the source of its promoting agent. Sequeira and Kelman (1962) also suggested that increased amount of IAA

in diseased tissue was mainly due to the pathogen based on i) the ability of the bacterium to produce copious amounts of IAA in culture and ii) the fact that levels of IAA continued to increase even after the plants had completely wilted. However, the validity of such arguments may be questioned on the basis of reported increase in IAA during initial stages of infection when the populations of the bacterium were relatively small. Braun (1954) has, on the other hand, postulated that the incipient tumor cells have, through induction, acquired the capacity to synthesize auxin and that attenuated bacteria alter the cells to a lesser degree. Genes responsible for IAA synthesis from crown gall cells have been isolated (ref. Kado, 1984; Rausch **et al.**, 1985).

In spite of a great deal of research, the physiology of auxins in tumors is still unsolved. Moreover, the various manifestations caused by auxin even in normal growth are not clear. Braun (1962) has drawn attention to the similarities of the abnormal histological and cytological features observed in tumor tissues and those found in normal. He considered that these features can be accounted for in terms of abnormal growth hormone physiology which is characteristic of the tumor tissue.

The uncontrolled or autonomous growth of tumor cells generally appears to reflect an abnormal hormone metabolism

(Tandon, 1985a). There are three major hypotheses for hyper-auxinity of gall tissues: i) increased synthesis, ii) decreased destruction, and iii) both increased synthesis and less destruction. The tumor tissues were shown to have an enhanced capacity for IAA biosynthesis either from tryptophan (Henderson and Bonner, 1952; Tandon and Arya, 1980a) or from other indolic precursor molecules (Atsumi, 1980a). Kehr and Smith (1954) reported that **Nicotiana glauca** x **N. langsdorffii** hybrids possess a higher auxin content and a more effective enzyme system for converting tryptophan to auxin as compared to the parents. Similarly, pith cells isolated from tumorous plants converted indole and tryptophan to IAA. On the other hand, pith tissue from non-tumorous plants grew poorly and needed, in addition, cytokinin for growth (Bayer, 1982). A relationship between tryptophan and auxin content in sunflower and tobacco crown gall cells in culture has been established (Atsumi, 1980b; El-Bahr **et al.**, 1985). However, the pathways of auxin biosynthesis in tumor tissues are poorly understood (Wood, 1972; Atsumi, 1980a).

As far as auxin catabolism is concerned some different points of view and paradoxes are raised in the literature. Since the discovery of IAA-oxidase (Galston **et al.**, 1953) in certain species, it became clear that IAA-oxidase destroys IAA by oxidation. Bitancourt (1949) reported the presence

of IAA inactivating enzymes in normal tissue but not in crown gall tumor tissue. The higher level of auxin was also found in the tumor cells resulting from a decreased destruction. The lower level of IAA-oxidase in tumor tissues of different plants as compared to their normal counterparts have been demonstrated (Sequeira, 1973; Wegen and Glase, has 1981). In contrast, several investigators have reported that the differences in IAA-oxidase and peroxidase activities in normal and tumor tissues did not account for the increased amounts of auxin (ref. Butcher, 1973). The necessity of auxin for the transformation of normal cells to tumor cells have been supported by a number of workers (Klein and Link, has 1955; Lippincott and Lippincott, 1975; Tandon **et al.**, 1976).

Biochemical changes from normal in **in vivo** tumor tissues have been reviewed (Kado, 1976; Lippincott and Lippincott, 1976). **In vivo** tumors of both tomato stems and sugarbeet roots showed clear evidence of a converting pattern in their comparative biochemistry as regards carbon, nitrogen, vitamin contents and enzymatic studies (Klein and Link, 1955). Vester and Anders (1960) reported that young plants of **N. glauca** x **N. langsdorffii** hybrid contained 50% more free amino acids than the parental species. Later on, Tso **et al.** (1962) observed significant differences in the concentrations of alkaloids, sugars, organic acids and amino acids. Differences in the contents of starch, soluble sugars,

carbohydrates, nitrogen, amino acid composition, aromatic amino acids and steroids have also been shown in insect/mite-incited plant galls (Shekhawat **et al.**, 1978; Tandon and Arya, 1979; Rohfritsch and Shorthouse, 1982; Dixon, 1983; Masahiro **et al.**, 1984).

Several differences have been reported in the activity of oxidative enzymes that are associated with crown gall tumor formation (Klein, 1965; Spurr **et al.**, 1965; Kado, 1976; Lippincott and Lippincott, 1976; Marijana, 1980). Spurr **et al.** (1965) showed increase in tyrosinase, ascorbic acid oxidase, chlorogenic acid oxidase and chlorogenic acid in tumor induction. Besides these, many other workers have studied the auxin metabolism and oxidative enzymes in plant tumor tissues (Bhatia **et al.**, 1967; Ahuja and Gupta, 1974; Purohit **et al.**, 1980; Wegen and Glase, 1981; Tandon and Arya, 1982; Joshi and Tandon, 1984).

The discovery of auxin protector substances in sunflower internodes inoculated with a virulent strain of **A. tumefaciens** by Stonier (1969) has given a new direction in the field of auxin metabolism in tumor tissues. Since then, the auxin protectors have also been reported in many abnormal growths (Atsumi and Hayashi, 1978; Haard, 1978; Tandon and Arya, 1980b). These substances are antioxidants which prevent the peroxidase-catalyzed oxidation of IAA. Stonier (1972) suggested that extremely high levels of auxin

protectors are sufficient to explain the autonomy even when crown gall tissues contain more auxin destroying enzymes.

The production of phenolic compounds, polyphenol oxidase and hydroxylases is an almost universal feature of disease. Various functions have been ascribed to phenolics like their role in disease resitance (Bell, 1981; Beart **et al.**, 1985; Rosenthal, 1986), as a food for phytophagous insects (Bernays **et al.**, 1983), and in the inactivation of proteins and enzymes (Anderson, 1968). Kehr and Smith (1954) have also shown the role of phenol and benzene derivatives in tumor formation in **Nicotiana.** Larger amounts of scopoletin and scopolin have been demonstrated in tumor-prone hybrids (**N. glauca** x **N. langsdorffii**) than in the parent species and it was found that these compounds could reduce the rate of IAA oxidation by peroxidase (Tso **et al.**, 1964).

Particular interest centers around the role of phenolic compounds in auxin metabolism. But how do they influence it? Whether or not their influence in **in vitro** and **in situ** would be the same? These are a few quests which have to be explored before their possible role in auxin metabolism can be concluded.

Plant tissue culture methods are used as a tool to understand not only normal but abnormal growths in plants

(Helgeson and Deverall, 1983). Plant tumor cells are known to show phytohormone autonomy in culture, apparently as a result of production of copious amounts of phytohoromones (Bayer, 1982) and other substance(s) or factor(s) which directly or indirectly affect the level of these phytohormones (Stonier, 1972; Atsumi and Hayashi, 1978; Bouckaert-Urban and Vendrig, 1981; Tandon and Arya, 1982). However, some authors do not support this hypothesis (ref. Kado, 1984).

The molecular and biochemical basis of tumorigenesis in crown gall have been extensively studied. However, such studies in insect/mite-induced galls are scanty. Therefore, an attempt was made to understand the biochemical and physiological changes that occur during gall formation in **Cinnamomum tamala** Fr.Nees plants, with special reference to auxin metabolism.