GENERAL DISCUSSION

CHAPTER - IX

GENERAL DISCUSSION

Studies on economically important trees as taken up in the present project have shown interesting results. Leaf spot disease was found to occur in all the trees studied. As a result of the disease, leaves of all trees except Mangifera indica and Eucalyptus globulus have shown significant post-infectional chemical changes. In this chapter, post-infectional changes, the phytoalexin response with special reference to the non-pathogen, the sensitivity of the different fungi, phytoalexin detoxification and the role of phytoalexins and other post-infectional compounds in disease resistance of all these plants are compared and discussed.

In vivo studies in Tectona grandis, Cassia fistula Morinda tomentosa, Syzygium cumini, Anogeissus latifolia and Madhuca indica have shown that post infectional changes occur in their chemical constitution. Such results are in line with results on many experiments in crop plants (Ramakrishnan, 1966; Reddy et al.,1976;Koti Reddy and Prasad 1979; Ghosh, 1979). As suggested by Allen (1959) many of the infection induced responses are general reactions of the plant for repair of damage.

In most of the plants analysed, an increase in concentration of phenolic compounds was noted. There appears to be an enhanced synthesis of phenols by the host through shikimic acid pathway and this corroborates the results of a number of workers who found that phenolic compounds accumulate in plant tissue as a result of infection. Tomiyama, 1963; Kuć, 1964; Mace, 1964; Rohringer and Sambroski,1967; Kosuge, 1969; Glazner,1982). An increase in the aglycones also is noted. This increase might arise from the release of phenols from their glucosides by β-glucosidase of either host or pathogen (Pridham, 1965). Since the hydroxyl group in phenol is the group responsible for the fungitoxicity, the exposure of hydroxyl groups makes the compounds more reactive and thus toxic to the invader. But Farkas and Kiraly (1962) pointed out that hydrolysis of glucosides may not be able to explain the unusually high accumulation of phenolic compounds in the diseased tissue although it may not necessarily be impossible to suppose that it could be a trigger for the increase of phenolic compounds.

The increase in concentration of phenolic compounds may be an instant reaction to infection and could possibly be involved with mechanisms of disease resistance. Plants differ in their response with regard to the quantitative changes in phenols in that, initially, there appeaed an increase in both susceptible and resistant varieties, with

symptom development, phenolics decrease in the susceptible cultivars while they accumulated in the resistant varieties (Bhattia et al., 1972; Neema 1979; Vidyasekaran, 1978; Reddy and Rao, 1979).

In addition to the quantitative changes in the phenols two more response mechanisms are observed in the present study. They are (1) altering the existing biosynthetic pathways and (2) initiating a new biosynthetic pathway.

(1) Altering the existing biosynthetic pathway: In this method, the chemical nature of a compound already existing within the plant is altered resulting in the production of more reactive compounds. The process of demethylation of the methylated flavonols is such a mechanism operative in Cassia and Morinda. Demethylation was also observed in Madhuca where myricetin was produced in place of the methoxylated myricetin. In Cassia, sterochemical changes of certain compounds have also been observed. Isomerisation was noted in Cassia, where p-coumaric acid of the healthy leaves was replaced by o-coumaric in diseased leaves. The fungicidal properties of o-coumaric acid are already explained in chapter 4. o-coumaric acid being a systemic fungicide would obviously resist further entry of the fungus. Thus isomerization could also be cited as one of the disease resistance mechanisms.

Methylated flavonols such as 3'4'-diOMequercetin and 4'OMekaempferol in Morinda and 4'-OMequercetin,4'-OMe-

kaempferol and 7,3'4'-triOMequercetin in Cassia were replaced by their hydroxylated compounds such as quercetin and Kaempferol. The presence of more hydroxy groups gives more reactive powers to these compounds and thus increases their toxicity. The hydroxylated compounds may need not always be produced by demethylation. In plants it is seen that the hydroxylated compounds are produced first which are later methylated or glycosylated. In such cases the availability of hydroxylated compounds may be due to the blockage of the methylation process. Therefore the mode of production of hydroxylated compounds can be resolved only after studying the biosynthetic pathways in plants.

In phytophthora infected potato tubers, Kosuge (1969) reported that one of the factors present in the resistant but absent in the susceptible tissues appears to convert phenolic compounds to form, more toxic to the fungus. In the absence of pre-infectional toxic compounds that can overcome the pathogen, the host plant produces more toxic post-infectional compounds to inhibit and thus restrict the growth of the pathogen. The pattern of demethylation is an excellent example of this mechanism. This is seen in plants belonging to two different families and also in the plants infected by two different fungi. This proves that unrelated plants may adopt a similar mode of resistance.

(2) New biosynthetic pathways: New pathways leading to the production of new compounds were seen in Tectona grandis,

Syzygium cumini, Eucalyptus globulus, Mangifera indica and Anogeissus latifolia. p-hydroxybenzoic acid, a xanthone and a glycoflavone were produced in Tectona, Mangifera and Anogeissus respectively. In Eucalyptus, a coumarin was produced while in Syzygium, a quinone was elaborated.

Though researches in phytoalexins gained great momentum of late, the results obtained involved more questions than answers. Bailey and Mansfield (1982) in their update on phytoalexins tried successfully to discuss them at length and solve them. But still more are to be desired and all the data obtained by the successive workers are to be examined to find out the answers to these querries.

The first major question is on the omnipresence of phytoalexins. From the present study it is apparent that the leaves of all plants do not produce phytoalexins, though those which do not produce phytoalexins may produce other post-infectional compounds. Phytoalexins have not been detected in Anogeissus and Madhuca. Failure to detect phytoalexins has been reported as absence of phytoalexins in Anogeissus and Madhuca. The post-infectional compounds from Anogeissus and Madhuca are not considered as phytoalexins, since the glycoflavone from infected leaves of Anogeissus was not produced when tested for phytoalexin response and myricetin from Madhuca was found not be antifungal. In these plants, phytoalexins would have been some other group of compound

which is not analysed. Production of phytoalexins in minute or undetectable quantities would make it difficult to isolate them and thus leads to the conviction that phytoalexins are not produced by these plants. To arrive at a better conclusion, a thorough study of the different parts of the plant other than the leaf should also be studied.

The situation is not different when one goes through the literature. It is difficult to ascertain whether a phytoalexin is not reported in a plant family due to the fact that it was not produced or detected or that no attempt was made. Failure to detect phytoalexins is attributed to several reasons. For e.g. Deverall (1977) described his failure to detect phytoalexin either in wheat leaves undergoing a hypersensitive reaction to Puccinia graminis or in cucumber leaves challenged with Colletotrichum sp. Later on Kuć and Caruso (1972) and Cartwright and Russel (1981) reported that phytoalexins are indeed produced by these plants.

The second question is on the activity of the phytoalexins in vivo. Phytoalexin production in response to a pathogen has been detected only in Eucalyptus, Morinda, Cassia and Syzygium. Eventhough phytoalexins are produced in these plants, leaf spot disease occur and thus the respective pathogens are successful. The occurence of the leaf spot disease in both the trees clearly show that successful pathogens overcome all of the defensive mechanisms of their hosts including

the ability of the plants to accumulate phytoalexins. Inspite of the fact that the phytoalexins in these plants have been proved to be antifungal by *in vitro* studies, they do not prevent the disease. This clearly indicates that the phytoalexins are not as active *in vivo* as *in vitro*. One possible reason is that they get detoxified.

There often appears to be a competition between a plant's ability to accumulate inhibitory concentrations of phytoalexins and the ability of microbes to detoxify phytoalexins. Successful pathogens obviously tip the balance in their favour. It has been suggested that the pathogenic capability of *Fusarium solani* f.sp. *phaseoli* on French bean hypocotyl may be its ability to detoxify the host's phytoalexin (Kuhn and Smith, 1979). Demethylation is another commonly observed metabolic alteration of phytoalexins. A number of fungi are known to demethylate pisatin in this way, e.g. pisatin was demethylated to 3,6a-dihydroxy-8,9-methylene-dioypterocarpan by *Nectria haematococca* (Mathews and van Etten, 1981).

Another possible reason is that, when the host fails to synthesis sufficient concentrations of phytoalexins to inhibit or prevent the onslaught of the pathogen, the infection becomes unabated and the disease starts. Experiments with *Madhuca* showed the presence of three compounds in traces on chromatograms of the diffusate from fungus treated leaves, which could not be isolated and identified due to the trace amounts present. In

some cases the microbes suppress the formation of phytoalexins such a, case is reported in susceptible potato cultivars where the formation of rishitin and phytuberin is suppressed by incompatible races of Phytophthora infestans (Vans and Kuć, 1971).

Delayed synthesis of phytoalexins could also cause diseases in plants. In bean, phaseollin occurs in infection sites of both the compatible and incompatible races of Colletotrichum lindemuthianum, but it occurs much earlier in the incompatible infection zone (Rahe, 1973).

It was interesting to find that when leaves of eight different trees were induced with spores of a common nonpathogen Fusarium solani, only Tectona grandis and Mangifera indica responded to the fungus. Strangely in this two trees, there was no phytoalexin response, when they were treated with the pathogenic fungus. Response to F.solani by only two plants indicate that the nature of response varies from plant to plant. Response may be either positive or negative, rapid or slow. Though all plants contain the genetic potential for resistance mechanism, it does not necessarily mean that all plants should respond to microbial attack.

Sensitivity of microorganisms to antimicrobial compounds vary from organism to another. In Tectona grandis is was found that a non-pathogen was more sensitive to p-hydroxybenzoic acid than the pathogen. Difference in sensitivity of Colletotrichum gleosporoides and Aspergillus niger to quercetin was also

significant. Quercetin was found to be more toxic to C. gleosporoides than A.niger. The toxicity of phenolic compounds is quite different to different parasites. For instance, chlorogenic acid was found not toxic to Ceratostomella fimbriata (Condon and Kuć, 1960). Phytophthora infestans (Sokolova et al., 1960) or to Gleosporium Kawakami (Wakimoto et al.,1959) but toxic to Helminthosporium carbonum (Kuć et al., 1956) and Thielaviopsis basicola (Hamptom,1962). Difference in sensitivity of fungi to phytoalexins such as capsidol (Stoessl et al., 1972), pisatin and phaseollin (Van Etten, 1973; pueppke and Van Etten, 1974; Smith et al., 1975) have been reported. However, Harris and Dennis (1976) reported that there was no clear difference in the sensitivity of potato tuber pathogens and non-pathogens to rishitin, phytuberin, anhydro-β-rotunol and solavetivone. Some of the pathogenic microorganisms are even insensitive to phytoalexins (Mansfield and Deverall, 1974; Ffleger and Herman, 1975 a,b).

The post-infectional compounds of Tectona, Cassia, Syzygium and Anogeissus could be implicated in disease resistance since most of them are fungitoxic. Though the fungitoxicity of the post-infectional compounds are not very high, it could possibly offer resistance to diseases upto a certain degree. They prevent secondary infection also. Phytoalexins from Eucalyptus, Syzygium, Cassia and Morinda may also be involved in disease resistance. It was observed

that only the young leaves of Eucalyptus globulus and Syzygium cumini yielded phytoalexins and not the older ones. Incidentally in both these trees, the leaf spot disease was found to occur in most of the mature leaves and very rarely on young leaves. The ability to produce phytoalexins is said to decline during senescence (Cruickshank and Perrin, 1965; Bailey, 1969) and it has also been proposed that fungal growth on young leaves may be restricted by phytoalexins produced by underlying cells in response to fungal metabolites diffusing from germinating spores (Bailey, 1969; Last and Warren, 1972). The role of phytoalexins in disease resistance have also been shown by biochemical and microscopical studies (Heath and Wood, 1971; Keogh et al.,1980).

Phytoalexins, being natural products and antimicrobial in nature, could be used as agents of disease control. Natural products have an advantage in that it may not have much sideeffects on the plants. Recently neem extract was reported to control tikka disease of groundnut in India. Though analogues of phytoalexins have not shown very promising results, more of field trials with different plants and pathogens could possibly prove its potential as a replacement for synthetic fungicides.

A few new host records have also been recorded in this thesis. Curvularia clavata Jain on living leaves of Tectona grandis Linn., Collectotrichum gleosporoides (Penzig) Penzig and Sacc. on living leaves of Morinda tomentosa Heyne and

Madhuca indica Gmel. and Aspergillus niger van Tieghem on living leaves of Cassia fistula have been reported for the first time.